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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy in a Chinese pedigree

A case report using brain magnetic resonance imaging and biopsy[☆]

Erhe Xu, Huiqing Dong, Milan Zhang, Min Xu

Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing 100063, China

Abstract

The present study enrolled a Chinese family that comprised 34 members and spanned three generations. Eight members were diagnosed with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and disease diagnoses corresponded with autosomal incomplete dominance inheritance. The primary clinical manifestations included paralysis, dysarthria, and mild cognitive deficits. Magnetic resonance imaging revealed diffuse leukoencephalopathy with involvement of bilateral anterior temporal lobes, in particular the pons. In addition, multiple cerebral infarction was identified in the proband. Sural nerve biopsy findings of the proband revealed granular osmophilic material deposits in the extracellular matrix, which were adjacent to smooth muscle cells of dermal arterioles. Screening exons 2–4 for NOTCH 3 mutations by direct sequencing did not reveal any abnormalities.

Key Words: cerebral autosomal dominant arteriopathy; dysarthria; granular osmophilic material; leukoencephalopathy; NOTCH 3; paresis; subcortical infarcts

Erhe Xu[☆], Doctor, Associate professor, Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing 100063, China

Erhe Xu and Milan Zhang contributed equally to this work.

Corresponding author: Huiqing Dong, Master, Associate professor, Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing 100063, China
shshtt@sina.com

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INTRODUCTION

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited cerebrovascular disease caused by a mutation of the NOTCH 3 gene^[1]. Clinical manifestations of CADASIL include migraine with aura, recurrent cerebral episodes, cognitive deficits, and psychiatric symptoms^[2]. MRI reveals leukoaraiosis with multiple lacunar infarcts in the deep white matter, predominantly in the frontal and temporal regions extending to the temporal pole^[3]. In Caucasian CADASIL patients, the primary clinical features include migraine, transient ischemic attacks, stroke, and cognitive impairment, and sensitivity of T2-weighted magnetic resonance imaging (MRI) for detecting anterior temporal abnormalities in Caucasian patients is 89–95%^[4-5]. The present study describes a Chinese CADASIL pedigree and presents radiological and electron microscopic findings from eight affected members.

RESULTS

Clinical manifestations of the proband and family affected members

The proband, a 41-year-old man, was

referred to the Neurology Department of Xuanwu Hospital with hemiparesis, dysarthria, and mild cognitive deficits. At 31 years of age, the patient experienced a slight weakness in the left leg that included numbness, which resulted in gait problems that did not interfere with a normal lifestyle. Over the next four years, symptoms progressively worsened and led to weakness in the left leg. Simultaneously, the right leg became weak and numb, and urinary retention and urgent micturition ensued, which persisted to time of the study. Over the last two years, the left hand became uncoordinated and mild dysarthria developed. Since then, the patient experienced mood disorders, as well as involuntary and frequent crying spells. The patient exhibited slight cognitive impairment over a ten-year period, which interfered with employment status, social interactions, and daily life. The patient did not suffer from migraine headaches during this period.

Past medical history revealed right central retinal artery occlusion and retinal hemorrhage at age 33, which was surgically treated. However, right-eye vision was also lost at time of study. There was no family history of diabetes, hypertension, or hypercholesterolaemia. Seven members in his family experienced similar syndromes (Table 1, Figure 1).

Table 1 Detailed clinical manifestations of affected family members

Family member	Sex	Age (year)	Age of onset (year)	Risk factor	Symptoms	MRI/CT
I 1	M	D (57)	48	Hypertension	Hemiplegia, cognitive deficits	Not done
II 1	F	D (58)	40	None	Bedridden, dysarthria, hydroposia bucking, involuntary and frequent crying spells, cognitive deficits	+
II 3	F	D (62)	48	None	Bedridden, dysarthria, hydroposia bucking, forced crying, diplopia, cognitive deficits	+ (supplementary Figure 1 online)
II 11	M	53	45	None	Weakness, hemiparesis, dysarthria, mild cognitive deficits	+
II 13	M	D (49)	39	None	Paralyzed in bed, dysarthria, hydroposia bucking, involuntary and frequent crying spells, diplopia, cognitive deficits	+
III 2	F	45	36	None	Hemiparesis, mild dysarthria and cognitive deficits	+
III 3	F	42	36	None	Hemiparesis, mild dysarthria and cognitive deficits	+
III 5 ^a	M	39	31	None	Hemiparesis, mild dysarthria and cognitive deficits	+

a: Proband; M: male; F: female; D: death; +: abnormality present; MRI: magnetic resonance image; CT: computed tomography.

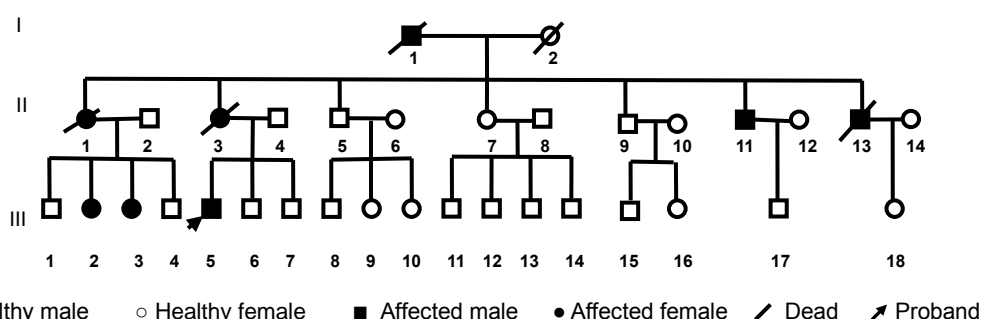


Figure 1 Pedigree chart of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy family from three generations with eight affected members.

Nervous system examinations of the proband

Neurological examination revealed normal vital signs, slight dysarthria, loss of vision, and direct and indirect light reflexes in the abducted right eye, with a 5-mm dilated pupil. Fundoscopic examination revealed optical nerve atrophy. Left-eye visual acuity and fields remained intact. Muscle mass was within normal limits. Strength was 4⁺/5 in the left leg, 5/5 in the left arm and right leg, and intact in the right arm. Deep tendon reflex was significantly increased, in particular on the left side. Extensor plantar responses were elicited on both sides. Gait was hemiparetic, with poor bilateral coordination. Patellar and ankle clonus was bilaterally present. Brown hemochromatosis and hypesthesia were noted on calf areas of both legs (supplementary Figure 2 online). Remaining physical examinations were unremarkable.

Other clinical examinations of the proband

Complete blood count, tests of hepatic and renal function, and measurements of lipoprotein A, electrolytes, glucose, thyroxine, vitamin B12, antinuclear antibodies and rheumatoid factor were all within normal limits. Serological examination for syphilis, human immunodeficiency virus, and tumor markers were negative. No abnormalities were found on cerebrospinal fluid analysis.

Previous brain computed tomography (CT) and MRI results revealed multiple infarctions, as well as multiple abnormal signals and bilateral pontine atrophy in the

head MRI. Hyperintense signals were noted in the corpus pons, genu of the callosum, left basal ganglia, corona radiata, and semiovale centrum on T2-weighted images, as well as symmetrical high signals in the bilateral temporal lobes (Figures 2A, B, D). A fluid-attenuated inversion recovery sequence revealed diffuse hyperintense signals in the subcortical and deep white matter (Figure 2C). Diffusion sequences produced negative results.

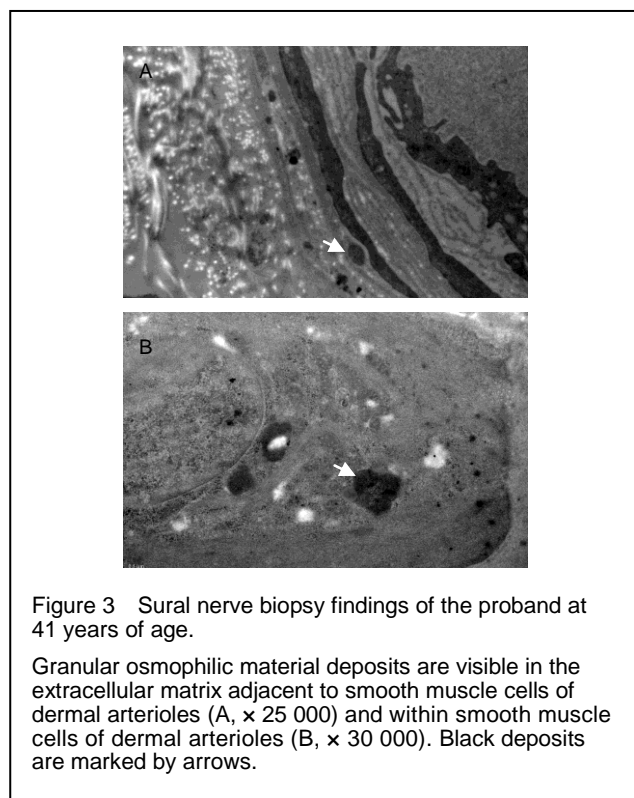
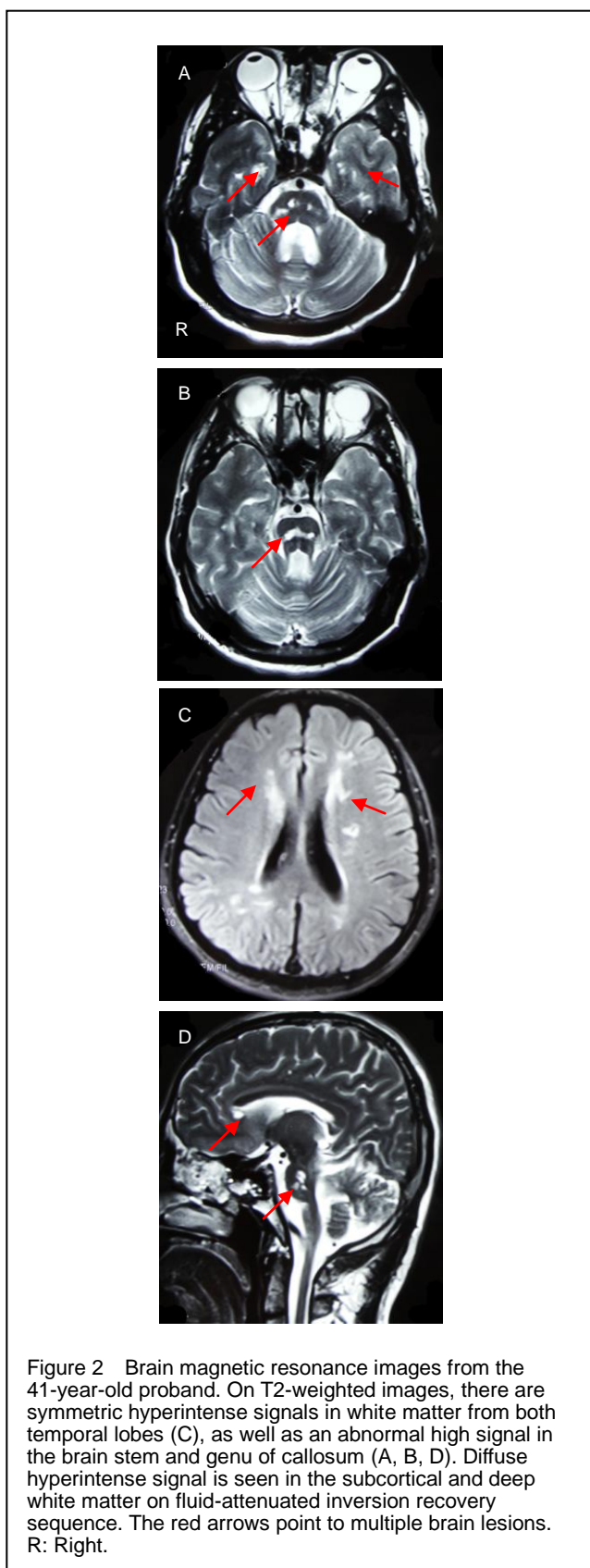
Sural nerve changes and NOTCH3 gene mutation analysis in the proband

A sural nerve biopsy was performed and the glutaraldehyde-fixed tissue was examined by transmission electron microscopy, revealing granular osmophilic material (GOM) deposits in the extracellular matrix adjacent to and within smooth muscle cells of dermal arterioles. The deposits were variable in size; the largest measured approximately 0.4–0.6 mm (Figure 3). Screening exons 2–4 for mutations of NOTCH 3 by direct sequencing produced negative results.

DISCUSSION

Family members presented with clinical manifestations of repeated stroke and cognitive deficits, which occurred in the absence of other vascular risk factors. MRI examinations revealed greater T2-weighted intensities in periventricular and subcortical white matter, including a

hyperintense signal in the corpus pons. These characteristics suggested CADASIL, which was confirmed by the presence of GOM on sural nerve biopsy.



CADASIL is characterized by five primary symptoms: migraine with aura, subcortical ischemic events, mood disturbances, apathy, and cognitive impairment^[6]. In Caucasian patients, 20–40% of patients exhibit migraine with aura, 60–85% of patients experience combined transient ischemic attack and ischemic strokes, approximately 20% of CADASIL patients experience severe depressive episodes, and 60% of patients > 60 years of age show signs of dementia^[7]. The present family experienced ischemic strokes, slight cognitive deficits, and mild depression, without migraines, which was similar to reports of mainland Chinese patients with low frequency migraine^[8]. Wang *et al*^[9] summarized 33 unrelated CADASIL patients from 12 provinces in mainland China, and analogically compared these patients to other Asian CADASIL patients from Japan^[10], Thailand^[11], Korea^[12], or Taiwan^[13], but only 5% of CADASIL patients also exhibited migraine. Genes or environmental factors might underlie low migraine frequency among Asian CADASIL patients. Notably, of the 89–95% reported cases in Caucasian populations^[5, 14], sensitivity of T2-weighted MRI for detecting anterior temporal abnormalities in mainland China patient cohorts was only 46%. Conversely, lacuna infarction in the brainstem is more common among Asian than among Caucasian CADASIL patients (64% vs. 45%)^[9]. In the present family, in addition to white matter hyperintensities in bilateral temporal lobes and periventricular subcortical regions, there was significant high signal in the pons, which was consistent with a high percentage of brainstem lesions in mainland China. In the last decade, more than 80 Notch 3 mutations have

been identified in > 400 families with CADASIL^[15]. Exons 4 and 3 are responsible for 85% of CADASIL patients of mainland Chinese descent, which differs from 47.6% Taiwanese patients with NOTCH3 mutations in exon 11^[9,13]. The present patients were screened for exons 2–4, but no mutation was detected. It is probable that the mutation belonged rather to one of the remaining 20 exons.

Although clinical manifestations are manifested in the brain, arteriopathy is also present in other organs, such as the spleen, liver, kidneys, muscle, aorta, and skin^[6]. The proband exhibited multiple brown hemoglobin deposits on the lower legs, which suggested extensive destruction of skin vessels. A sural nerve biopsy detected GOM deposits in the extracellular matrix adjacent to, and within, smooth muscle cells of dermal arterioles. GOM composition is not well known, but a previous study showed that the NOTCH 3 ectodomain might be the major component^[16]. The method that detects GOM from a skin biopsy can be used to diagnose CADASIL with 100% specificity, but variable sensitivity (45–90%), in different studies^[17].

At present, there is no treatment of proven efficacy for CADASIL, either for the disease or for the main symptoms. Treatment is entirely pragmatic and involves control of cerebrovascular risk factors, as well as supportive management for migraines, mood disorders, seizures, and cognitive impairment^[6]. Migraines with aura rarely require prophylactic treatment, because the frequency of attacks remains low in most patients. If required, typical prophylactic drugs, such as anti-epileptic drugs or β -blockers, can be used. According to anecdotal reports, acetazolamide has been found to be effective^[18-20]. The prevention of ischemic attacks is based on typical preventive measures for non-cardioembolic ischemic stroke: use of anti-platelet drugs rather than anti-coagulants, as well as treatment of vascular risk factors. Antihypertensive drugs are prescribed for hypertension^[21-22]. With regard to cognitive impairment, a randomized, controlled trial in CADASIL tested the efficacy of donepezil in patients with cognitive impairment. Donepezil had no effect on primary endpoint (cognitive subscale of the vascular Alzheimer's disease assessment scale), whereas several measures of executive functions improved^[23]. Rehabilitation, physiotherapy, psychological support, and nursing care are important for this severe, chronic, debilitating disease, as well as genetic counseling, particularly for asymptomatic members at risk of carrying the mutation. Because of multiple, small vessels, there is extensive destruction. Therefore, it is important to ensure that acetylsalicylic acid and warfarin are not used by the patient.

SUBJECTS AND METHODS

Design

A familial, clinical case report.

Time and setting

Data collecting and sural biopsy were performed at Xuanwu Hospital, China between April and October 2010. NOTCH 3 gene mutation screening was performed at Peking University First Hospital, China from May to July 2010.

Subjects

The proband was admitted to the Neurology Department of Xuanwu Hospital in April 2010, together with seven members from his family with similar syndromes of hemiparesis, dysarthria, and mild cognitive deficits. Diagnostic criteria for CADASIL included the presence of mutations in the NOTCH 3 gene and/or deposits of GOM or NOTCH 3 ectodomain in blood vessels^[4,24]. Informed consent was obtained from all family members, and the study was performed in accordance with the *Administrative Regulations on Medical Institution*, issued by the State Council of China^[25].

Methods

Mutation screening of the NOTCH 3 gene

Mutation screening of the NOTCH 3 gene from the proband was performed at Peking University First Hospital, China. Genomic DNA was extracted from peripheral venous blood of the left upper limb using standard protocols. Exons 2–4, as well as the flanking sequences, of NOTCH 3 gene were amplified by PCR. A 25- μ L PCR reaction contained 0.2 mM each primer, 100 mM each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM Mg²⁺, 0.1% Triton X-100, 1 U Taq DNA polymerase, and 50–100 ng genomic DNA. PCR products were directly sequenced after purification using BigDye (Applied Biosystems, Foster City, CA, USA) on an automated sequencer (ABI 3730; Applied Biosystems).

Pathological examination

A sural nerve biopsy examination was performed in the proband. The sural nerve samples were initially fixed in 2.5% glutaraldehyde, followed by 1% buffered-osmium tetroxide, dehydration in ascending grades of ethanol, and Epon embedding. Semi-thin sections of the sural nerve were cut and stained with toluidine blue to identify arterioles for thin sectioning. Thin sections (1.5- μ m thick sections) were double-stained with uranyl acetate and lead citrate, and then examined under a transmission electron microscope (Phillips CM100; Amsterdam, The Netherlands).

Author contributions: Erhe Xu and Milan Zhang collected clinical data from the family members, and drafted the manuscript. Min Xu performed the sural nerve biopsy and detected the GOM deposits. Huiqing Dong coordinated the study and revised the manuscript; he was also responsible for authorization.

Conflicts of interest: None declared.

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Supplementary information: Supplementary data associated with this article can be found, in the online version, by visiting www.nrronline.org, and entering Vol. 7, No. 3, 2012 after selecting the "NRR Current Issue" button on the page.

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