



# Cerebrotendinous xanthomatosis with peripheral neuropathy: a clinical and neurophysiological study in Chinese population

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**Background:** Cerebrotendinous xanthomatosis (CTX) is an inborn disorder of bile acid metabolism caused by deficiency of sterol 27-hydroxylase (*CYP27A1*) gene. CTX-related peripheral neuropathy has rarely been discussed in Chinese population. Here, we reported 6 CTX cases and performed a literature review focused on CTX with neuropathy to summarize its clinical and neurophysiological features.

**Methods:** All clinical data of 6 CTX cases were collected, and 21 reported Chinese CTX patients (including this study) were reviewed and summarized.

**Results:** Clinical manifestations of 6 CTX cases showed great heterogeneity. Cognitive decline, spastic paraplegia, cerebellar ataxia and advanced bulbar palsy were common neurological disorders, often accompanied by non-neurological signs like xanthomas, cataract, diarrhea and pes cavus. Dentate nuclei hyperintensity with or without hyposignal is a valuable MRI hallmark. Pooling our patients and literature review together, peripheral neuropathy was predominant sensorimotor demyelinating type in Chinese population, with an evident length dependent pattern and increased vulnerability in motor nerves. Demyelinating and axonal degeneration tend to exist in severe neuropathy. Three novel mutations including c.1055C>A; c.432T>G; c.472T>G were identified in *CYP27A1* and predicted to be pathogenic. Oral CDCA therapy could ameliorate some of the existing symptoms and provide clinical stability, but it could not cease disease progression completely.

**Conclusions:** Our study broadens the phenotype and mutation spectrum of CTX. Patients with cognitive decline, spastic tetraparesis, cerebellar ataxia and bulbar palsy, should be highly suspicious of CTX even no xanthomas disclosed. Peripheral neuropathy was predominant sensorimotor demyelinating type in Chinese population, with mixed axonal and demyelinating type in severe cases. Three novel likely pathogenic mutations including c.1055C>A; c.432T>G; c.472T>G were identified in *CYP27A1*.

**Keywords:** Cerebrotendinous xanthomatosis (CTX); peripheral neuropathy; China; *CYP27A1*; clinical feature

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## Introduction

Cerebrotendinous xanthomatosis (CTX), is a rare autosomal-recessive disorder of bile acid metabolism, caused by an inborn deficiency of the mitochondrial sterol 27-hydroxylase encoded by *CYP27A1* gene (OMIM \*606530) (1). The lack of sterol 27-hydroxylase blocks the pathway from cholesterol to chenodeoxycholic acid (CDCA), with diversion then to cholestanol, resulting in accumulation of cholesterol and cholestanol in tissues throughout the body, particularly in the central nervous system (CNS), lens, and Achilles tendons (2,3).

Clinically, the hallmarks of CTX include infant-onset chronic intractable diarrhea, juvenile-onset bilateral cataracts, tendinous xanthomas, and progressive neurologic dysfunction characterized by pyramidal signs, cerebral ataxia, cognitive impairment and peripheral neuropathy (4,5). Neonatal CTX was easily overlooked and often had a worse prognosis (6,7). Diagnosis was often made in adults or teenagers when typical clinical spectrum was fully developed with a long diagnostic delay (8). Elevated plasma cholestanol aid in diagnosis but was not available to detect in China (9). Treatment with CDCA (750 mg/d) normalizes the level of the bile acid precursor, helps maintain cholesterol homeostasis and improves neurological and non-neurological symptoms (10,11).

Peripheral neuropathy was a frequently reported feature of CTX from sporadic cases and series determined by EMG or biopsy. However, there remains controversies regarding whether neuropathy in CTX is demyelinating or axonal in origin. The most prominent neuromuscular feature documented in Dutch CTX patients was sensory motor polyneuropathy and muscle was not specifically involved (12). As for Chinese CTX cases, the neuropathological and electrophysiological features were discussed rarely but seemed to reflect a potential different profile from foreign cases.

In China, CTX was likely far under-diagnosed based on a recent epidemiological study and the enormous population (6). Poor recognition and unavailable cholestanol detection might be the cause. Considering the treatable nature and potential high incidence, we reported our own cases to broaden clinical and genetic spectrum of CTX, and reviewed literature to discuss CTX-related peripheral neuropathy in China. We present the following article in accordance with the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-2746>).

## Methods

### *Subjects and clinical evaluation*

Our six CTX patients were diagnosed from January 2018 to December 2019. Their medical records were carefully reviewed. Brain and Achilles's tendon magnetic resonance imaging (MRI), laboratory tests, electromyography (EMG) analysis results were collected. Subjects were followed up every 6 months by routine appointment in out-patient clinic. For patient lost follow-up, information before the last follow-up was used. The diagnostic criteria in this study included clinical symptoms, genetic analysis and exclude differential diagnosis, adapted by the criteria previously established by a Japanese nationwide survey (13). The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Qilu Hospital, Shandong University (No. KYLL-2016-229). Written informed consent was obtained from the patient for publication of this study and any accompanying images.

### *Pathological examination*

Right sural nerve biopsy was performed in 3 patients. The stained frozen and semithin sections were investigated under light microscopy and electron microscopy individually.

### *Gene analysis*

The mutations in the *CYP27A1* gene using PCR and DNA sequencing were detected in all patients' blood samples (Running-Gene, Beijing, China). Conformational studies and familial segregation analysis were commonly performed.

### *Literature review*

To delineate features of peripheral neuropathy in Chinese CTX cases, we reviewed all published cases and series study in Chinese population from PubMed during 2002 to 2020. Together 21 genetic proven cases with detailed data of EMG findings were collected and summarized in this study (including our cases) (14-22). For each case reported, we documented the clinical symptoms, EMG profiles, genetic data and nerve pathological descriptions if information were available.

### *Statistical analysis*

Statistical collating and analysis were performed using

SPSS 23.0 (SPSS Inc., Armonk, NY, USA). Clinical data of each category were listed directly and proportions of each category were also displayed.

## Results

### *Clinical features*

All 6 patients came from different non-consanguineous families. There were 5 males and 1 female patients. Their detailed clinical information was listed in *Table 1*. Only Patient 3 had positive family history, whose sister presented the same symptoms as him. All patients were diagnosed in their 20s or 30s with a diagnose delay ranging from 10 to 27 years. Four patients (66.67%) presented chronic diarrhea since early childhood as initial symptoms. Lower limbs weakness and mental retardation was first manifestation of the other 2 cases. Five patients developed bilateral cataracts before 15 years old and 3 of them underwent bilateral cataract extraction. Pes cavus, a commonly hereditary sign, was found in 5 patients. Patient 4 had undergone orthomorphia for pes cavus three times. He also suffered frequent bone fracture because of unsteady gait and osteoporosis. Achilles's tendon xanthomas were disclosed in only 3 patients. The presence of characteristic xanthomas seemed not related to the severity of clinical symptoms. For example, Patient 2 with typical tendon xanthomas showed slight neurological dysfunction while Patient 3 presented typical CTX manifestations like mental decline, pyramidal signs, ataxia and severe neuropathy without any xanthomas. Furthermore, Patients 4 and 6 developed gallstones and related abdominal pain, the latter underwent cholecystectomy. All patients exhibited cognitive decline confirmed by decreased MMSE scores. Spastic paraplegia and cerebellar ataxia were frequently found in 5 patients. Only 2 patients presented epilepsy. Three patients developed bulbar palsy. Symptoms of patient 1 were aggravated after puberty at 25 years old. All but patient 2 showed an elevated blood total bilirubin and no patients harbored hypercholesterolemia.

### *Neuroimaging findings*

All 6 patients underwent brain MRI illustrated by *Figure 1*. The main abnormal findings included bilateral symmetrical hyperintensity lesions of bilateral dentate nucleus, cerebral peduncle and posterior limb of the internal capsule on T<sub>2</sub>W and FLAIR imaging. Atrophy of the cerebrum and

cerebellum can also be noticed, generally mild or moderate. Patients 1, 4, 5 showed some decreased signal on T<sub>1</sub>W/FLAIR in bilateral cerebellar hemisphere. Brain MRA in Patient 1 confirmed slight premature atherosclerosis despite normal serum cholesterol concentrations. Three patients (Patients 1, 2 and 6) evaluated ankle MRI and showed fusiform thickening and heterogeneous signals in the Achilles tendon.

### *Electrophysiological and pathological findings*

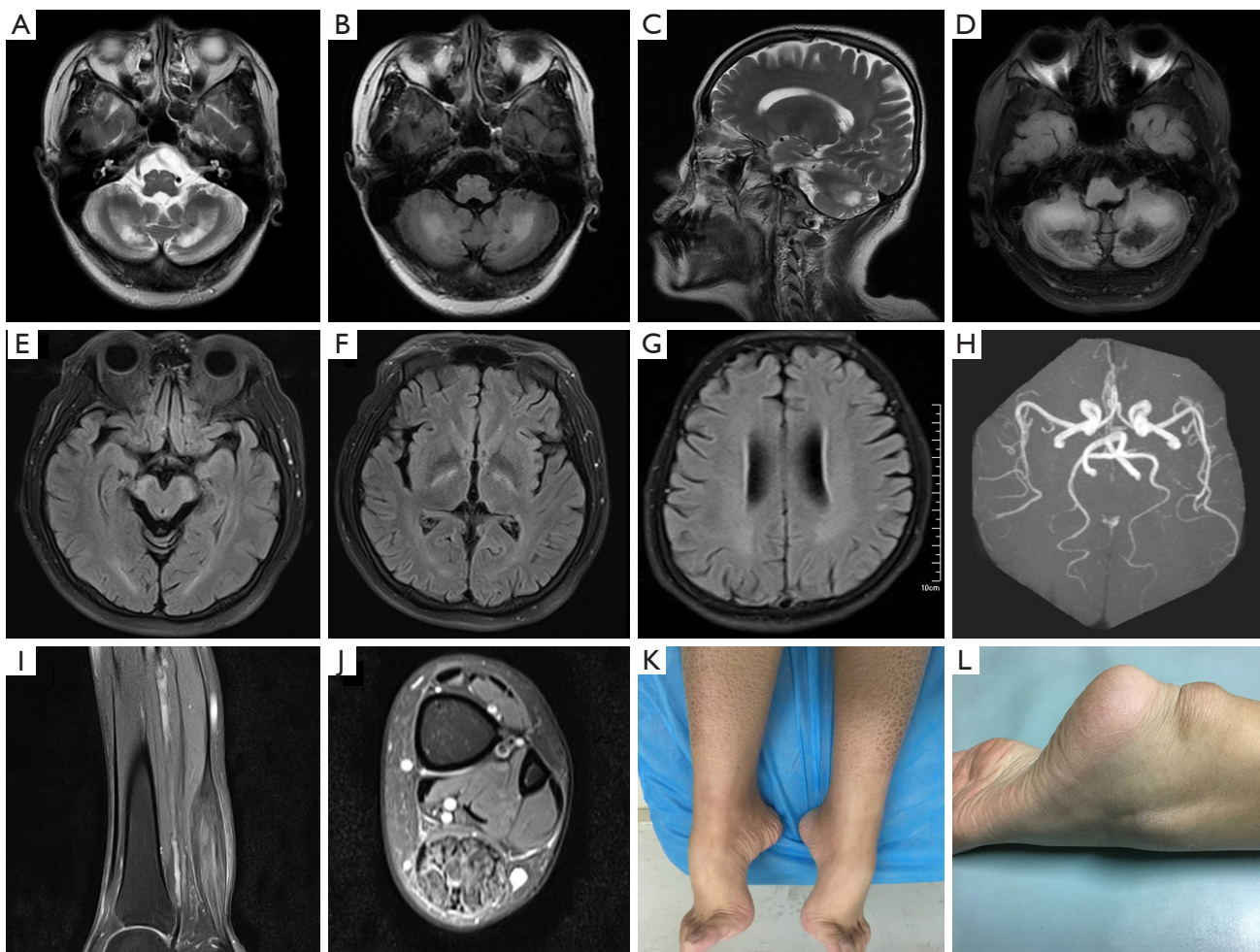
Electrophysiological study revealed motor or motor sensory demyelinating predominant polyneuropathy in all 6 cases. Decreased nerve conduction velocity (NCV) and prolonged F response latencies were prominent features, particularly in lower limbs' motor nerves, indicating a length dependent pattern. Slowed motor NCV below 75% normal level and prolonged distal latencies over 130% indicated a conspicuous primary demyelinating process. In Patient 4 and 5, no motor or sensory conduction could be elicited in lower limbs. In some patients, length dependent axonal involvement was also existed, characterized by a decreased amplitude of sensory and motor nerves. As for needle EMG, Patients 1, 4, 5 disclosed evidence of moderate chronic neurogenic injury, characterized by decreased recruitment patterns, increased polyphasic waves, prolonged duration and giant amplitude of motor unit action potentials (MUAPs).

Three patients underwent sural nerve biopsy with confirmed peripheral neuropathy (*Table 2* and *Figure 2*). Nerve biopsy of patient 2 showed slightly loss of myelinated fibers, about 10%. Some fibers with relatively thick myelin sheaths can be seen, exhibiting massive red in MGT staining (*Figure 2A*). In Patient 3, semithin sections showed a decreased density of large myelinating fibers by about 20%. Extensive onion bulbs were formed around demyelinated fibers, suggesting a chronic process of demyelination and remyelination. Regeneration cluster of small myelinated fibers (sprouting) can be frequently seen (*Figure 2C,D*). As for Patient 4, large-diameter, myelinated fibers were markedly decreased by about 40%. Some fibers presented thin myelin sheath. Semithin section revealed evidence of acute axonal degeneration with myelin bodies and chronic degeneration with clusters of regenerating fibers (*Figure 2B*). Onion bulb was not apparent. In accordance with electrophysiological data, sural nerve biopsy showed histological features of demyelinating and axonal peripheral neuropathy, with myelin sheath more seriously involved in Patient 3 and axon

**Table 1** Clinical characteristics of 6 patients in this study

Characteristics	Patient					
	1	2	3	4	5	6
<b>General symptoms</b>						
Gender	F	M	M	M	M	M
Age of diagnosis (years)	29	35	22	26	29	30
Age of onset (years)	2	25	2	2	2	3
Family history	-	-	+	-	-	-
Diarrhea (years)	2	-	2	2	2	-
Cataracts (years)	4	-	15	6	+	10
Xanthomas (years)	7	35	-	-	-	7
Osteoporosis (years)	-	-	-	26	-	-
Pes cavus	+	+	+	+	-	+
Others	-	-	Ichthyosis	-	Crinosity	Gallstones
<b>Neurological symptoms</b>						
Mental retardation/cognitive impairment (years)	6	35	6	6	19	3
Psychiatric abnormality (years)	24	-	-	-	-	-
Spastic paraplegia (years)	24	-	3	20	19	30
Cerebellar ataxia (years)	24	-	3	20	19	30
Epilepsy(years)	-	-	3	-	-	29
Peripheral neuropathy (years)	+	+	+	+	+	+
Dysarthria (years)	24	35	-	-	19	30
Muscle weakness (years)	-	35	+	+	-	30
Dysphagia (years)	29	35	-	26	-	-
<b>Brain T2/FLAIR hyperintensity</b>						
Dentate nuclei	+	+	+	+	+	+
Cerebral and cerebellar atrophy	-	-	-	+	-	+
Capsula interna	+	+	-	-	+	-
Brain stem	+	+	-	-	+	-
Cerebral white matter	+	-	-	+	-	+
<b>Biochemistry</b>						
Cholesterol	ND	ND	ND	ND	ND	ND
Bilirubin	↑	Normal	↑	↑	↑	↑
Cholesterol	Normal	Normal	↑	Normal	Normal	Normal
Others	-	-	Vitamin D↓	TG↑	-	-
<b>Electrophysiological study</b>						
Axonal neuropathy	+	-	+	++	++	+
Demyelinating neuropathy	++	+	++	++	++	++
<b>Mutations in CYP27A1</b>						
Allele1	c.1263+1G>A	c.472C>T	c.1263+1G>A	c.1263+1G>A	c.1214G>A	c.379 C>T
Allele2	c.1420C>T	c.432T>G	c.1055C>A	c.1004C>T	c.1004C>T	c.1263+1G>A
Outcome	Improved	Not improved	Not improved	Improved	Stable	Improved

F, female; M, male; ND, not detected; +, present; -, absent; numbers stand for the onset age of each symptom; ↑, elevated; ↓, decreased.



**Figure 1** MRI and feet images of our CTX patients. T2 weighted image and FLAIR image of Patient 1 show hyperintensity with mixed low signal intensities in bilateral dentate nucleus and surrounding cerebellar white matter (A,B,C). Patient 4's FLAIR image reveals low signal intensity consistent with cerebellar vacuolation in the same area (D). FLAIR image of patient 2 shows high signal intensities in bilateral corticospinal tract including cerebral peduncles (E) and posterior limbs of internal capsule (F). FLAIR image of Patient 2 and 5 show posterior periventricular white matter lesions (E) and focal subcortical white matter lesion (G) respectively. Brain MRA of Patient 1 reveals premature atherosclerosis (H). Patient 2's T2-weighted FS MR image shows a fusiform thickening of Achilles tendon with mixed signal intensity (I), and spotted high signal in the thickened tendon mass (J). Patient 3 showed typical pes cavus and ichthyosis-like skin change while Patient 1 reveals enlargement of the Achilles tendon (K,L).

also seriously damaged in Patient 4. Electron microscopy confirmed onion bulbs formation in Patient 3.

### **Molecular genetic analysis**

The gene sequencing of all 6 patients revealed 8 variants in *CYP27A1* gene, including 3 novel mutations (Table 3). Patient 1,4,5 and 6 identified 5 reported pathogenic mutations and confirmed the diagnosis, including. c.1263+1G>A, c.1420C>T, c.1004C>T, c.1214 G>A and

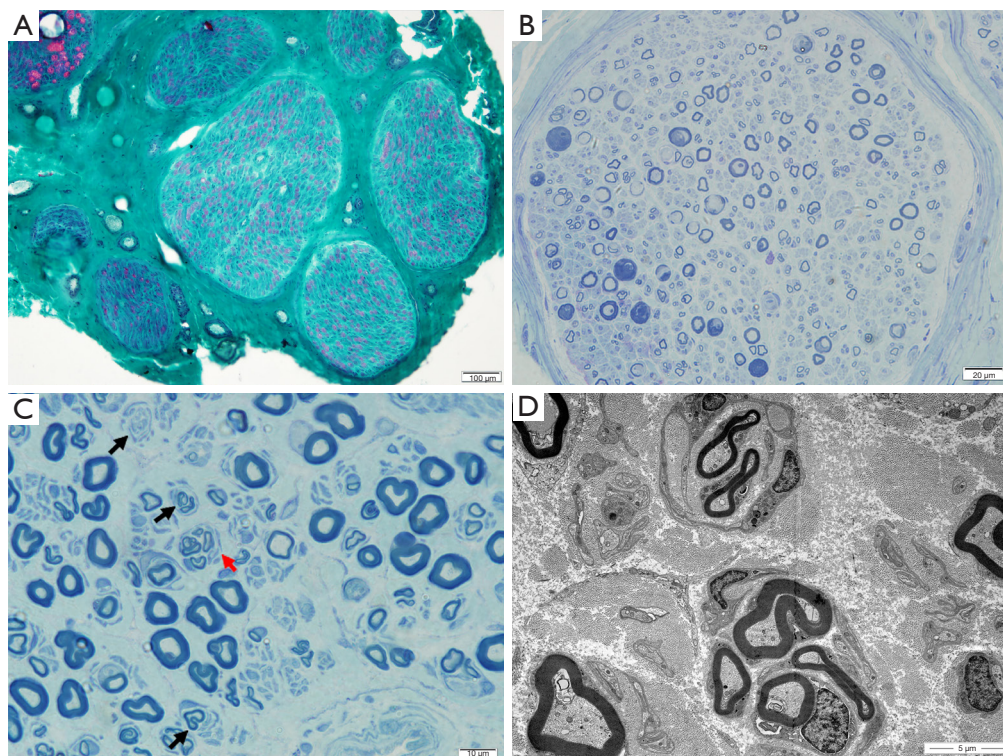
c.379C>T in *CYP27A1* (9,23-27). Among these detected variants, c.1263+1G>A seemed to be a very hot variant since 4 of our patients had detected this allele.

Patient 2 was identified compound heterozygous of novel mutations c.432 T>C (p.Y144X) and c.472 C>T (p.R158C). c.432 T>C (Y144X) generated a premature termination codon and caused protein truncation or nonsense-mediated mRNA decay. It was classified as likely pathogenic according to ACMG guidelines, although registered as a likely benign variant in ClinVar database. c.472 C>T (p.R158C)

**Table 2** Sural nerve biopsy findings in 3 CTX patients

Variables	Patients in this study		
	Patient 2	Patient 3	Patient 4
Age at biopsy (years)	35	22	26
Decreased density of large myelinating fibers	10%	20%	40%
Thinly myelinated axons	–	+	+
Onion bulbs	–	+++	++
Myelin digestion chamber (MDC)	±	±	+
Thick myelin sheaths	++	–	–
Myelin bodies	–	+	++
Regeneration clusters of smaller myelinated fibers	–	+	–

–, absent; ±, dubious; (+) to (+++) indicates mild to severe.



**Figure 2** Nerve biopsy findings in Patients 2–4. (A) Slightly reduced myelinated fibers and fibers with thick myelin sheath (Patient 2, MGT  $\times 100$ ); (B) myelinated bodies and many thin myelinated fibers (Patient 4, semithin toluidine blue  $\times 400$ ); (C) large amount of onion bulbs formations (black arrow) and a regeneration cluster of small myelinated fibers (red arrow) (Patient 3, semithin toluidine blue  $\times 1000$ ); (D) onion bulbs formation (Patient 3, electron microscopy  $\times 5,000$ ).

was a missense mutation located outside the functional domain interacting with two protein cofactors of sterol 27-hydroxylase. However, p.R158 residue was well-conserved in many species and substitution of this residue by a novel

cysteine was predicted to alter the structure of the enzyme. It was predicted as deleterious according to PolyPhen-2 (score: 1.000) and disease causing in Mutation taster with the probability of  $>0.99$ . These two novel mutations were likely

**Table 3** Novel mutations detected in 6 CTX patients and their pathogenesis classification

Genomic alteration	Co-segregation	Nucleotide alteration	Amino acid alteration	SIFT	PolyPhen-2	Mutation taster	Conservation	1000 genomes	ExAC browser	HGMD	ACMG interpretation
chr2-219674476	Yes	c.432T>G	p.Y144X	-	-	DC	-	0	0	No	Likely pathogenic
chr2-219676970	Yes	c.472C>T	p.R158C	Deleterious	Probably damaging	DC	Highly conserved	0	4/121216	No	VUS
chr2-219678781	Yes	c.1055C>A	p.S352X	-	-	DC	-	0	0	No	Pathogenic

SIFT, <http://sift.jcvi.org>; Polyphen-2, <http://genetics.bwh.harvard.edu/pph2/>; Mutation Taster, <http://www.mutationtaster.org>; 1000 Genomes, <http://www.1000genomes.org>; ExAC Browser, <http://exac.broadinstitute.org>; HGMD, <http://www.biobase-international.com/product/hgmd>. DC, disease causing.

**Table 4** Treatment response evaluation in 6 patients during a 2-year follow-up

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Diagnostic age	29	35	22	26	29	30
Treatment	CDCA	UDCA + atorvastatin	CDCA	CDCA	CDCA	CDCA
Duration of treatment	2 years	1 year	1.5 years	2 years	0.5 year (poor adherence)	1 year
Follow up						
Improvement	Slow responses, stagger gait, psychiatric symptoms	No	Diarrhea, weight gain, speech	Diarrhea, weight gain, energetic feeling	No	Unstable gait, cognitive impairment
Stabilization	Dysarthria, spastic paraplegia	Recent-memory loss, slow response	Unsteady gait, cognitive impairment		Excessive drooling, non-verbal, diarrhea	
Deterioration	Dysphagia, occasionally choking, lower limb weakness	Mild dysphagia and dysarthria	No	Gait imbalance, accidental falls, dysphagia, muscle atrophy, tremor	Gait imbalance	No
mRS score at baseline/follow-up	2/2	1/2	2/2	3/4	2/2	2/1

CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid.

to cause CTX when compound heterozygous.

Patient 3 carried compound heterozygous of a novel mutation c.1055C>A (p.S352X) and a reported variant c.1263+1G>A. The former was a nonsense mutation predicted as disease causing according to mutation taster. The region p.Ser352 was located very closed to the adrenodoxin cofactor binding site (residues 384–398) and the premature stop codon resulted in truncated protein and further loss of the functional domain (28). This allele was not found in ClinVar database. As nonsense mutations were often pathogenic, it was considered to cause disease when in combination with another severe deficiency allele like c.1263+1G>A.

### Follow-up

All patients were advised to initiate CDCA at a dose of 750 mg/d as well as low-cholesterol diet after diagnosis. Since CDCA was not so available in China, Patient 2 used UDCA and atorvastatin instead (Table 4). Treatment duration was at least half a year and patients were followed up for 2 years. Data was missing in Patient 2 after 1-year follow-up. Even though 4 patients with good adherence of CDCA relieved some of the symptoms or remained clinically stable, some symptoms continued progressing chronically. No adverse effect was observed. Patient 1 showed improvement in slow

responses, stagger gait and psychiatric symptoms after 6 months. But she became occasionally choking and complained lower limb weakness at 2-year follow-up. Patient 2 still presented slow response and memory loss, and developed mild dysphagia and dysarthria under UDCA and atorvastatin. Patient 3 remained clinical stable in gait imbalance and cognitive impairment. His diarrhea relieved, weight gained and speech became more understood. Patient 4 also showed decreased diarrhea, weight gain and energetic feeling at one-year follow-up. Unfortunately, he continued to deteriorate in ataxia and suffered accidental falls. Muscle atrophy in lower limbs, dysphagia and tremor were newly developed symptoms. Patient 5 took CDCA for a short duration due to poor adherence. Unsteady gait continued to decline. Patient 6 improved his ataxia gait and recognition function (MMSE 8 to 11) after taking CDCA for a year. Evaluated modified Rankin Scale (mRS) at last follow-up were unchanged in 3 patients, decreased in 1 patient, and increased in 2 patients. It seemed CDCA had a limited effect once neurological symptoms were fully established since it could not prevent neurological progression especially in bulbar palsy. Unfortunately, none of our cases reevaluated NCV/EMG.

#### *Literature review of CTX patients with peripheral neuropathy in China*

To achieve a better understanding of CTX-related peripheral neuropathy, we reviewed literature and summarize 21 cases from PubMed, including our cases. Their clinical data and description of peripheral neuropathy were analyzed (Table 5). Demyelinating dominant peripheral neuropathy was found in 10 cases, axonal in 3 cases and mixed axonal and demyelinating polyneuropathy in 3 cases. Four cases reported by Chen (14) showed normal NCV and case reported by Gao (20) didn't mention the specific type of peripheral neuropathy. Overall, 4 cases were motor predominant and 12 cases were sensorimotor. Thirteen cases showed a length dependent pattern with distal part more seriously involved. The profile of neuropathy features seemed not related to the genotype, disease duration or onset age. In Chinese patients, demyelinated polyneuropathy in a length dependent pattern was a prominent feature of CTX-related neuropathy. Mild NCV abnormal tended to be demyelinated changes like slowed conduction velocity and prolonged distal latency, and once the neuropathy fully developed, severe demyelinating as well as axonal changes often coexisted, leading absence response in the lower limbs.

## Discussion

CTX was once considered as an exceedingly rare hereditary disease with an estimated rate of less than 5/100,000 worldwide (29), but seemed far under-diagnosed based on a recent epidemiological study (3). The great heterogeneity of clinical spectrum and long period between symptoms emerged made early diagnosis a challenge. Based on our patients, xanthomas was not an indispensable sign because patients with typical neurological disorders could be in absence of tendon mass. Another indicative symptom was early-onset diarrhea. It seemed more frequently reported by Caucasian than Chinese population in a rate of 92% vs. 3.8% (22). However, our cases showed a relatively high proportion of chronic diarrhea (4/6), which could be relieved by long-term administration of CDCA. This discrepancy might result from genetic background differences, recall bias or inadequate medical history collecting. Furthermore, pes cavus was very common in some hereditary status and should be carefully examined in CTX. In addition, many CTX patients underwent cataract surgery before reaching the final diagnosis, providing ophthalmologists an opportunity to aid in early diagnosis. It was estimated that children with bilateral cataracts showed an astonishing prevalence of CTX of 1.8% (30,31). Failure to realize those non-neurological symptoms were part of a broad clinical spectrum of an underlying metabolic disorder was a main cause of diagnostic delay. It is important for clinicians from different specialties to create great awareness of and better screening for CTX. Early identification strategy described by Duell was helpful in judging the diagnosis (32).

Cognitive impairment, pyramidal signs, cerebral ataxia and peripheral neuropathy were prominent neurological disorder. As disease progressed, dysphagia, dysarthria, ambulatory difficulties and muscle weakness continue to aggravate, and finally lead to total dependence or even death, to some extent very similar to end-stage ALS (amyotrophic lateral sclerosis). Coincidentally, CYP27A1 had been identified as a possible candidate gene for ALS in a large and comprehensive genome-wide screening (33). It seemed plausible that CYP27A1 deficiency causes a reduction of liver X receptor (LXR) ligands and a decrease of motor neuron (MN) survival signaling (33-38). Specific cholestenic acids might act as potential MN protectors and neuroinflammation modulators, shedding light on the management of neurodegenerative disease. In our cases, CDCA can alleviate some of neurological and non-neurological symptoms but the effect was somehow



Table 5 Clinical and electrophysiological data of CTX patients with peripheral neuropathy in Chinese population

Case	Distribution/ year	Mutation 1	Mutation 2	AAO/ AAE	G	Dia	Cog	Cat	Xan	Neu	Ata	Pym	Bul	Mus	Nerve conduction study	EMG	Sural nerve biopsy	Reference
1	Mainland/2020	c.1263+1G>A	c.1420C>T	2/29	F	2	6	4	7	+	24	24	24	-	M demyelinated pnp, LD	Chronic denervation	ND	This study
2	Mainland/2020	c.472C>T	c.432T>G	25/35	M	-	-	-	25	+	-	-	35	35	M demyelinated pnp, LD	Normal	Demyelinated	This study
3	Mainland/2020	c.1263+1G>A	c.1055C>A	2/22	M	2	6	15	-	+	3	3	-	22	S-M demyelinated dominant pnp, LD	Normal	Demyelinated	This study
4	Mainland/2020	c.1263+1G>A	c.1004C>T	2/26	M	2	6	16	-	+	20	20	26	26	S-M demyelinated axonal pnp, LD	Chronic denervation	Demyelinated and axonal	This study
5	Mainland/2020	c.1214G>A	c.1004C>T	2/29	M	2	19	19	-	+	19	19	19	-	S-M demyelinated dominant pnp, LD	Chronic denervation	ND	This study
6	Mainland/2020	c.1263+1G>A	c.379C>T	7/30	M	-	7	10	7	+	7	-	-	-	S-M demyelinated dominant pnp, LD	ND	ND	This study
7	Mainland/2017	c.1537C>T	c.1477-2A>C	3/48	M	-	-	-	33	-	43	43	46	43	Normal	Normal	ND	(14)
8	Mainland/2017	c.410G>A	c.410G>A	30/35	M	-	-	-	30	-	-	33	-	-	Normal	Normal	ND	(14)
9	Mainland/2017	c.410G>A	c.379C>T	42/43	F	-	-	-	42	-	-	-	-	-	Normal	Normal	ND	(14)
10	Mainland/2017	c.562C>T	c.1214G>A	34/37	F	-	-	-	37	-	-	34	36	37	Normal	Normal	ND	(14)
11	Mainland/2018	c.379C>T	c.1174G>A	8/27	M	-	11	8	11	-	23	23	23	11	Normal	NK	ND	(15)
12	Taiwan/2011	c.1333C>T	c.1263+1G>A	NK/24	M	-	+	+	+	+	+	+	-	-	S-M axonal demyelinated pnp, LD	Chronic denervation	ND	(16)
13	Taiwan/2011	c.1333C>T	c.1263+1G>A	NK/23	M	-	+	+	+	+	+	+	-	-	S-M axonal pnp, LD	Chronic denervation	ND	(16)
14	Taiwan/2011	305delC	305delC	NK/37	F	-	6	6	6	+	+	+	-	-	S-M axonal pnp, LD	Chronic denervation	ND	(16)
15	Taiwan/2011	305delC	305delC	NK/32	M	-	7	7	+	+	+	+	-	-	M dominant axonal pnp, LD	Chronic denervation	ND	(16)
16	Mainland/2011	c.73delG	c.369_375del GTACCCA	7/36	F	7	13	7	27	+	7	7	35	27	S-M demyelinated axonal pnp	NK	Demyelinated and axonal	(17)
17	Taiwan/2002	c.379C>T	c.1263+1G>A	6/34	M	-	6	14	20	+	20	20	33	20	M demyelinated pnp, LD	NK	NK	(18)
18	Mainland/2007	c.379C>T	c.1420C>T	6/42	F	-	6	37	37	+	6	6	33	41	S-M demyelinated dominant pnp	Chronic denervation	Demyelinated	(19)
19	Mainland/2018	c.379C>T	c.1214G>A	6/28	M	-	+	-	37	+	37	37	-	37	S-M pnp, LD	ND	ND	(20)
21	Mainland/2020	c.435G>T	c.562C>T	1/49	F	10	1	-	39	+	24	24	-	-	S-M demyelinated dominant pnp, LD	NK	ND	(21)
22	Mainland/2019	c.435G>T	c.571C>T	36/38	M	-	-	-	36	+	37	37	43	-	S-M demyelinated dominant pnp	NK	ND	(22)

G, gender; AO, age at onset; AD, age at examination; Dia, diarrhea; Cog, cognitive impairment; Cat, cataracts; Xan, xanthomas; Ata, ataxia; N, neurology; Pym, pyramidal signs; Bul, bulbar palsy; Mus, muscle weakness; M, motor; S, sensory; pnp, peripheral neuropathy; LD, length dependent; ND, not done; NK, not known; +, present; -, absent. Numbers indicate the age when symptom emerged.

limited. Early diagnosis is important because initiating supplement of CDCA at asymptomatic stage could prevent disease progression (5). Treatment after 25 years old had worse outcome and limited improvement in ambulation and cognition (32). Hopefully, measures aiding in early diagnosis were in sight. For example, dried blood spot (DBS) screening assay excellently distinguished newborn CTX patients based on different ratio between cholestanetetrol glucuronide (tetrol) and taurochenodeoxycholic acid (t-CDCA) (39). Prominence of sulfated and sulfated/glucuronidated BALc conjugates in urine is new biomarker facilitating rapid diagnosis in infancy (40).

Brain MRI showed extremely high diagnostic value for CTX. Hyperintensity in dentate nuclei was probably the initial result of abnormal lipid storage, while hyposignal in T<sub>2</sub>FLAIR indicated vacuolation and calcification caused by cholestanol-induced apoptosis, predominantly in the late stage (41,42). Diverse involvement of cerebellum indicated different state of natural disease progression, which started in the dentate nucleus and then extended to the surrounding white matter (43). Patient 1,4 and 5 in our series showed dentate hypointensity changes, and they all developed to bulbar palsy—a clinically advanced sign, consisting with the above hypothesis. In some cases, diffuse or focal hyperintense ischemic-like lesions (T<sub>2</sub>W/T<sub>2</sub>FLAIR) were found in subcortical and periventricular white matter, reflecting a process of demyelination or gliosis in the CNS (43). Premature atherosclerosis detected by MRA might be a result of reduced reverse cholesterol transport mediated by CYP27A1 deficiency.

In the literature, etiology of peripheral neuropathy in CTX was a matter of debate. Reported CTX cases utilized different methods-NCV/EMG/or nerve biopsy/or muscle biopsy to confirm and describe different type of neuropathy. Some authors believed the peripheral neuropathy was demyelinating in nature with evidence of recurrent demyelination and remyelination (44-46). Others considered length-dependent axonal neuropathy as the major pathological abnormality (12,47-49). However, large published case series and literature review tend to support the latter point of view. For example, a study from Netherlands included 58 patients and established axonal neuropathy in the majority of patients in whom an EMG was performed (5). A research from Italy identified polyneuropathy in 26 CTX patients and 76.9% of them was predominantly axonal (38.4% sensory-motor axonal and 38.4% motor axonal) (50). And another study in which 10 patients were observed through EMG and sural nerve

biopsies found that the most prominent neuromuscular abnormality was sensorimotor axonal polyneuropathy (12). Furthermore, researchers from Taiwan focused on CTX-related neuropathy and concluded axonal degeneration was the most common type (16). In contrast, demyelinating dominant neuropathy was mostly reported by sporadic cases or small series. In this study, CTX-related neuropathy from 21 Chinese cases exhibited a different picture. Our study results were consistent with those of Pilo *et al.* (51) that sensorimotor demyelinating neuropathy was the prominent feature. When lesions of peripheral neuropathy were relatively slight, motor nerves were more vulnerable to demyelinate in a length dependent pattern. Axonal and demyelinating degeneration in sensory and motor nerves often coexisted in severe cases.

It is proposed that polyneuropathy in CTX can be divided into 3 types: axonal, demyelinated and mixed polyneuropathy (19). In our cases, patient 2 and 3 belonged to the 2<sup>nd</sup> type. Patient 4 showed both myelin and axon affected and pertained to be the 3<sup>rd</sup> type. Another 2 Chinese cases undergone nerve biopsy showed demyelinated and mixed polyneuropathy respectively (17,19). No genotype and neuropathy type relationship was found. The mechanism of divergence remains unclear. Several reasons need to be considered. First, sample size was relatively small compared to broad case series. Second, different genetic background between mainland China and Taiwanese and Caucasian populations might be a reason. In addition, pathophysiological mechanism underlies the development of neuropathy. Brimming with cholesterol, myelin sheath insulates and facilitates axonal conduction but also becomes vulnerable in this sterol metabolic disorder. Replacing large amounts of cholesterol with cholestanol that have different structure and electrical conductivity would conceivably have significant effects upon function of myelin sheath. As cerebral white matter was primarily involved in CNS (42,52), we considered it plausible to include a demyelinating process in peripheral neuropathy. Besides, CYP27A1 is a mitochondrial enzyme related to energy metabolism. Like other metabolic disorders, CTX has features of distal, sensorimotor axonal polyneuropathy. Either axons or myelin involved predominantly varies as disease progress, exhibiting different types of polyneuropathy. Also, inter-practitioner inconsistency in performing and analyzing NCV/EMG data should also be taken into consideration. Further studies with large sample size are needed.

There are several shortcomings in our study. First, reported

CTX cases might be subjected to recall bias. The variation of inter-practitioner must relate to differences in NCV test performance. Secondly, the present 6 cases have not detected serum cholestanol level because this test was not available at present in China. Thirdly, no functional verification of the three novel mutations was done. Further research and follow-up studies are needed to conform these results.

## Conclusions

Patients presented with cognitive decline, spastic paraplegia, cerebellar ataxia should be suspicious of CTX, especially accompanied with xanthomas, cataract, diarrhea and pes cavus. Dentate nuclei hyperintensity is a valuable MRI hallmark. CTX-related peripheral neuropathy was predominant sensorimotor demyelinating type, with an evident length dependent pattern and increased vulnerability in motor nerves. Demyelinating and axonal degeneration tend to coexist in severe neuropathy. Three novel mutations c.1055C>A, c.432T>G, c.472T>G are detected and predicted pathogenic, providing new data for mutation spectrum and molecular diagnosis of CTX. Oral CDCA therapy early on can provide clinical stability and may also ameliorate existing symptoms, but it could not cease disease progression completely.

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## Footnote

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*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. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Qilu Hospital, Shandong University (No. KYLL-2016-229). Written informed consent was obtained from the patient for publication of this study and any accompanying images.

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