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Clinical, biochemical, and molecular insights into Cerebrotendinous Xanthomatosis: A nationwide study of 100 Turkish individuals

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ABSTRACT

Objective: Cerebrotendinous xanthomatosis (CTX) is an inherited metabolic disorder characterized by progressive neurologic and extraneurologic findings. The aim of this retrospective, descriptive study was to explore the time of presentation and diagnosis, and to expand the phenotype and genotype of CTX, based on a nationwide and comprehensive series of patients in Turkey.

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CYP27A1
Diagnostic delay

Methods: The demographic, clinical, biochemical and genotypic characteristics of the CTX patients were reviewed. Data on molecular analysis, age of onset and diagnosis, diagnostic delay, neurologic and extra-neurologic symptomatology, results of plasma cholestanol levels, brain magnetic resonance imaging and electromyography at the time of diagnosis were reviewed.
Results: 100 confirmed CTX patients from 72 families were included. The mean age at diagnosis was 28.16 ± 14.28 years, and diagnostic delay was 18.39 ± 13.71 years. 36 patients were diagnosed in childhood. Frequency of intention tremor ($p = 0.069$), peripheral neuropathy ($p = 0.234$) and psychiatric manifestations ($p = 0.396$) did not differ between two groups, demonstrating the high rate in pediatric patients. Three adult patients showed a milder phenotype without neurologic involvement. Seven patients had normal plasma cholestanol levels despite neurological impairment. Sequencing of the *CYP27A1* gene revealed 25 different variants, with a novel c.671_672del variant not previously described in literature.
Conclusion: Based on the observations of this Turkish CTX cohort, it is emphasized that the true prevalence of CTX is probably underestimated and that it has a wide spectrum of clinical phenotypes even without neurological impairment. In children, abnormal cerebellar findings, peripheral neuropathy and psychiatric findings associated with intellectual disability have been suggested as warning signs to avoid diagnostic delay. In cases of clinical suspicion, molecular analysis is recommended despite normal plasma cholestanol levels, as severe neurologic involvement may occur in CTX patients without elevated cholestanol levels.

1. Introduction

Cerebrotendinous xanthomatosis (CTX, MIM: #213700) is an autosomal recessive metabolic disorder caused by biallelic pathogenic variants in the gene *CYP27A1* (OMIM: *606530), which encodes the enzyme sterol 27-hydroxylase. Since the mitochondrial sterol 27-hydroxylase enzyme plays a major role in the classic and alternative bile acid synthesis pathways, reduced enzyme activity leads to impaired bile acid production, particularly of chenodeoxycholic acid (CDCA) and to a lesser extent of cholic acid (CA). The interrupted negative feedback effect of CDCA on cholesterol 7α-hydroxylase then accelerates the resulting main metabolic abnormalities; an increase in 7α-hydroxylation pathway is observed, while the depletion of 27-hydroxylated and carboxylated cholesterol products is remarkable. Abundant resulting metabolites, such as 5α-cholestanol, 7α-hydroxycholest-4-en-3-one, 7α,12α-dihydroxycholest-4-en-3-one (7α,12α-diHCO), 7α-hydroxycholesterol (and potentially lack of other metabolites) are thought to contribute to pathogenesis and neurotoxicity in CTX [1,2].

The clinical phenotype of the disease includes both neurologic and extraneurologic findings. Developmental delay, intellectual disability, cerebellar, pyramidal and extrapyramidal signs, dementia, psychiatric manifestations, peripheral neuropathy, seizures and spinal involvement have been listed as neurologic findings of the disease. Juvenile cataract, tendon xanthomas, cholestatic jaundice and persistent diarrhea are the most commonly reported extraneurologic findings, along with skeletal system manifestations such as osteoporosis, cardiovascular findings and respiratory system involvement [1,3]. According to the current literature, the clinical manifestations of CTX in children are dominated by extraneurologic findings, whereas in adults, neurologic findings are predominant. CTX generally presents with cholestatic jaundice, chronic diarrhea and intellectual disability in childhood, while cataract and epilepsy contribute to the clinical phenotype in adolescence and neuropsychiatric changes usually occur after the second decade of life [4,5]. However, the natural history of the disease is not yet fully understood; neuropsychiatric manifestations have been reported in pediatric CTX cohorts [6–8], and adult patients without neurologic involvement diagnosed with isolated xanthomas have also been reported [9,10].

The standard treatment for CTX is CDCA at a dose of 5–15 mg/kg/day for pediatric and 750 mg/day for adult patients, as exogenous CDCA inhibits the bile acid synthesis pathway and can stabilize or even improve the neurologic and extraneurologic findings [11]. However, the timing of the start of treatment plays a pivotal role in the prognosis of the disease [12]. Initiation of the treatment before the onset of neurologic findings can result in a favorable outcome, while delaying diagnosis and treatment can lead to a poorer prognosis and worsening of symptoms despite treatment [13,14].

The estimated prevalence of CTX is evaluated to be 3–5/100000, though its prevalence varies by country and ethnic group, and nationwide population studies reflecting the real prevalence data is widely lacking in the literature [15–17]. Recognition of the disease can be challenging because the clinical phenotype is heterogeneous and nonspecific neurologic symptoms can mimic other neurodegenerative disorders. As a result, most of the CTX patients remain likely under- or misdiagnosed; diagnostic delay has been reported approximately two decades after the onset of the initial symptoms [4,5,18].

Here, we report the time of presentation and diagnosis, emphasize the family screening, and expand the natural history, clinical and molecular phenotypes from a nationwide and comprehensive series of CTX patients of Turkey.

2. Materials and methods

2.1. Patients

This is a retrospective descriptive study designed in accordance with the current revision of the Declaration of Helsinki and includes patients with CTX diagnosed and followed up in 21 major reference centers specialized for neurometabolic diseases in Turkey. Data from patients diagnosed with CTX between January 1997 and January 2024 were analyzed. The study was approved by the local ethics committee (E-83045809-604.01.01-873441). Patients with a clinical diagnosis of CTX confirmed by elevated plasma cholestanol level measurement and/or *CYP27A1* gene sequencing and living in Turkey were included in the study. Sequence variants were classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) [19].

2.2. Data collection and evaluation

The demographic, clinical, biochemical and genotypic characteristics of the CTX patients were reviewed. The following items were recorded from the medical data: Sex, geographic origin, molecular analysis, consanguinity, age of onset and diagnosis, initial symptom, neurologic and non-neurologic symptoms associated with the disease. Most patients had reported various symptoms up to the time of diagnosis, but the first symptom with which the patient consulted a physician for examination was considered as the initial symptom. The diagnostic delay time was calculated as the time between the date of molecularly or biochemically confirmed CTX diagnosis and the initial symptom. Three or more loose stools within 24 h were considered as diarrhea, and diarrhea lasting longer than 14 days was considered as persistent diarrhea [20]. However, changes in stool frequency were also considered when evaluating diarrhea in the first months of life, as normal stool frequency varies widely among infants [21].

A detailed neurologic and systemic examination, initial plasma cholestanol level, brain magnetic resonance imaging (MRI) and electromyography (EMG) at the time of diagnosis were also reviewed. The results of neurocognitive tests administered by a licensed neuropsychologist, including the Wechsler Intelligence Test for Children IV (WISC-IV) and Wechsler Adult Intelligence Scale (WAIS) were recorded. In the patients with epilepsy, the results of the electroencephalogram (EEG) examination were noted.

The upper normal range for plasma cholestanol was assumed to be below 7 μg/ml [22,23]. The age limit of 19 years was used to define the age groups of children and adults, taking into account the recommendations of the World Health Organization.

In the patients who underwent bone mineral density (BMD) measurement, bone mass was assessed according to the guidelines of the International Society for Clinical Densitometry (ISCD). In the pediatric group, a BMD *Z*-score of less than or equal to −2.0 SD was considered "low bone mass". Osteoporosis was defined as the combination of a BMD Z-score ≤ -2 and a history of clinically significant fracture [24,25]. In the adult group, the assessment criteria were similar to those of the pediatric group for premenopausal women and for men younger than 50 years. However, T-scores were preferred for postmenopausal women and men aged 50 years and older. A BMD T-score ≤ -2.5 was considered as "osteoporosis" [26].

All the examinations mentioned above referred to the findings at the time of diagnosis; follow-up examinations were not taken into account.

2.3. Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were displayed as arithmetic means±standard deviations; categorical variables were displayed as frequencies or percentages. The normal distribution of data was evaluated with a Kolmogorov–Smirnov test. Analysis of the normally distributed quantitative variables was made by independent sample *t*-test. Pearson's chi-square test was used for the comparison of categorical data. A p level ≤ 0.05 determined statistical significance.

3. Results

3.1. Demographic data of the patients

One hundred clinically and genetically confirmed CTX patients (56 males and 44 females) from 72 families were included in the study. Eighty-three patients had consanguineous parentage. The mean age of the diagnosis was 28.16 ± 14.28 years. Thirty-six patients (36%) were diagnosed in childhood and 64 were diagnosed in adulthood. Mean age of onset was 9.76 ± 9.69 years and the diagnostic delay was reported as 18.39 ± 13.71 years. Considering the geographical origin of the patients, the Black Sea (35%) and Central Anatolia (22%) were the regions where the disease was most frequently diagnosed. The distribution of the CTX patients according to the geographical origin is shown in Fig. 1.

The clinical, biochemical and molecular findings of the 100 patients were summarized in the Supplementary Material.

3.2. Clinical phenotype of the patients

Cataract (22%), febrile and/or afebrile seizures (15%), persistent diarrhea (14%) and intellectual disability (13%) were reported as the most common presenting symptoms. Isolated dystonic movement disorder (1%) and myopathy (1%) were the rarest presenting signs reported by two patients. Prior to CTX diagnosis, one or more misdiagnoses were reported in 48% of patients, mainly intellectual disability (13%) and unspecified epilepsy (15%). While 42% of patients had only one misdiagnosis by the time of CTX diagnosis, five patients had two and one patient had three different misdiagnoses. Data on the distribution of initial symptoms and misdiagnoses prior to CTX diagnosis are shown in Fig. 2.

Intellectual disability (78%) and cataract (75%) were the most frequently reported clinical manifestations occurring during the course of the disease up to CTX diagnosis. Ophthalmologic findings other than cataract were reported in 10 patients (refractive error in five, uveitis in two, optic disc drusen in one, and vision loss due to glaucoma in two patients). Symptoms likely related to cerebellar involvement were frequent falls while walking and/or clumsiness, reported by 69% of patients. At least one of the psychiatric manifestations other than autism occurred in 61% of patients, with mood/affective disorders (42%), behavioral/personality disorders (39%), and anxiety (34%)

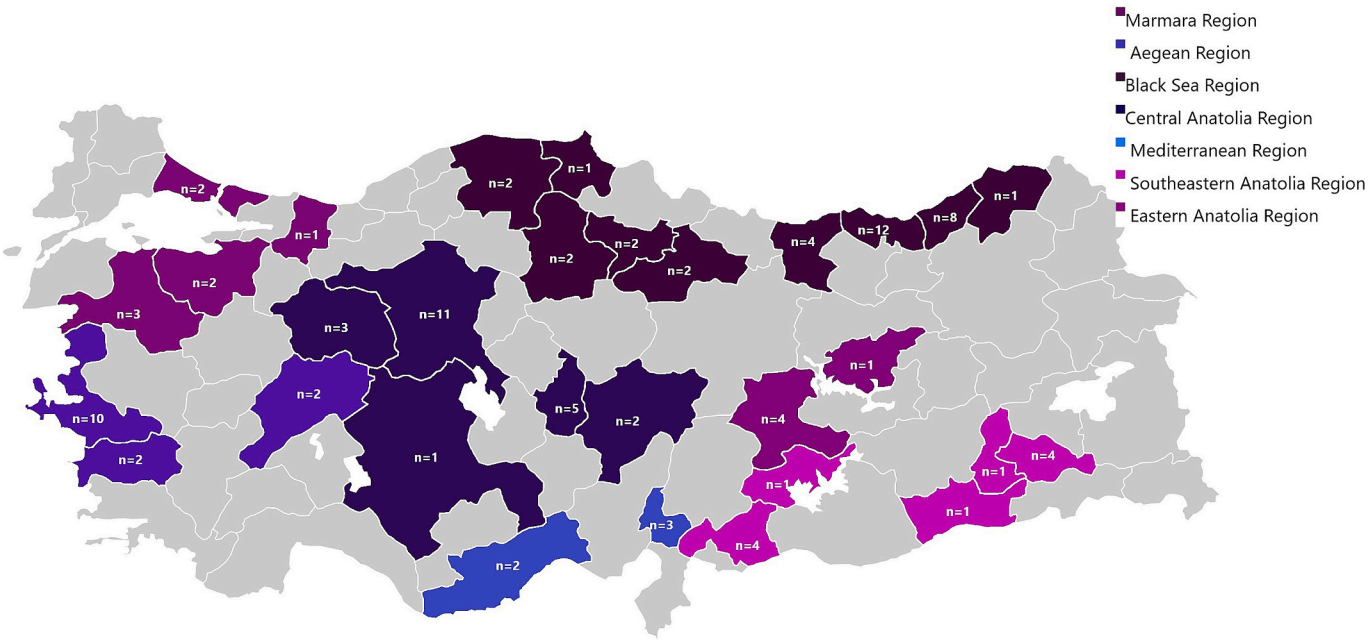


Fig. 1. The distribution of the CTX patients according to the geographical origin in Turkey.

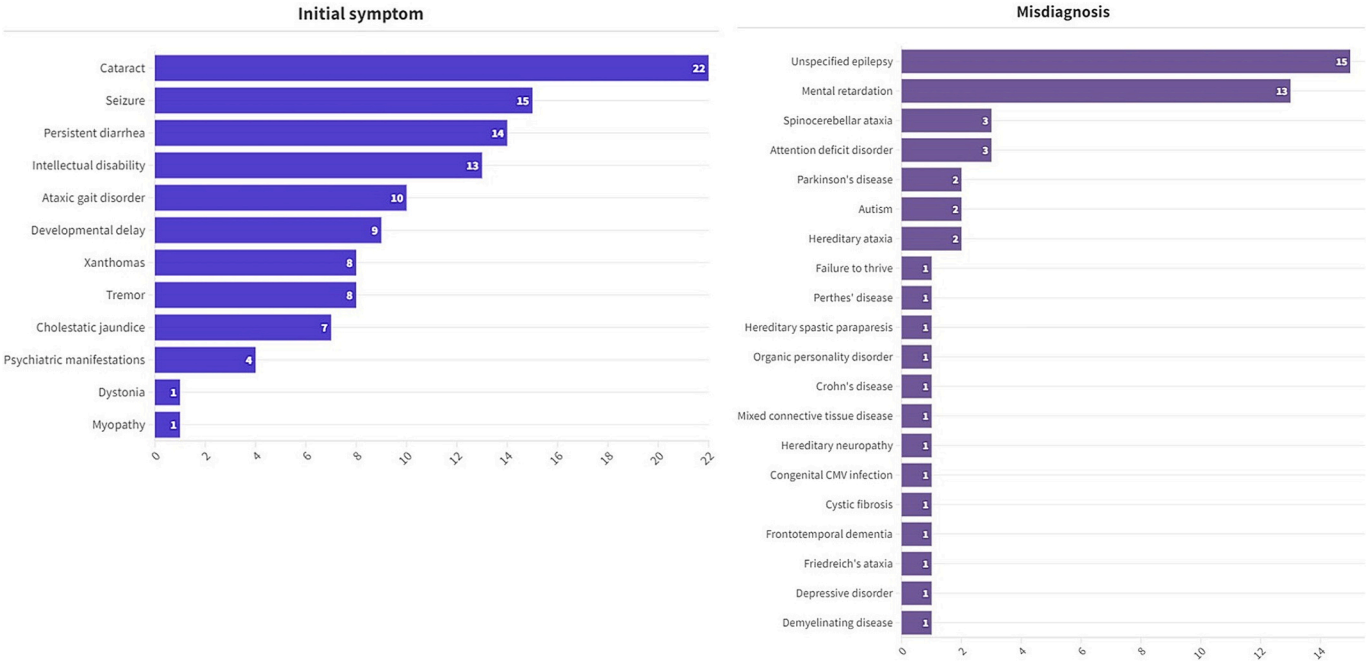


Fig. 2. Data on the distribution of initial symptoms and misdiagnoses prior to CTX diagnosis ($n = 100$ patients).

predominating. Four patients had signs consistent with autism. Dementia was diagnosed in 19% of patients. 43% of patients had experienced at least one febrile or afebrile seizure prior to CTX diagnosis. Antiepileptic treatment was initiated in 32 of these patients. An EEG analysis was available in 23 of 32 patients, which revealed abnormalities in 15 patients. Persistent diarrhea (40%) and developmental delay were the other common clinical symptoms. Delays in language skills (37%) were more common than delays in motor development (31%). Atherosclerosis (1%), cholestatic jaundice (7%) and liver disease (6%) were rare findings. Data on the distribution of the patients' clinical characteristics of the entire study cohort and distribution of positive clinical findings according to the age groups are shown in Fig. 3.

As data on the mean age at onset of the individual CTX- relevant symptoms were not available for all patients included in the study, information on the natural history of the overall clinical symptoms was insufficient. According to the available data, persistent diarrhea was observed in early childhood (mean age at onset: 4.82 ± 3.65 years, data from 11 of 40 patients), followed by intellectual disability in the first decade of life (mean age at onset: 8.88 ± 3.17 years, data from 18 of 78 patients). Cataract was also observed early in the course of the disease (mean age at onset: 15.15 ± 9.67 years, data from 26 of 75 patients). Symptoms probably related to cerebellar involvement were first observed at the beginning of the second decade (mean age at onset: 23.9 ± 14.75 years, data from 13 of 69 patients).

At the time of diagnosis, the neurologic examination was dominated by cerebellar signs. Eighty-four patients had at least one of the cerebellar signs, with ataxia (73%), intention tremor (55%) and dysarthria (54%) predominating. Nystagmus was found in 15 patients. Signs of pyramidal

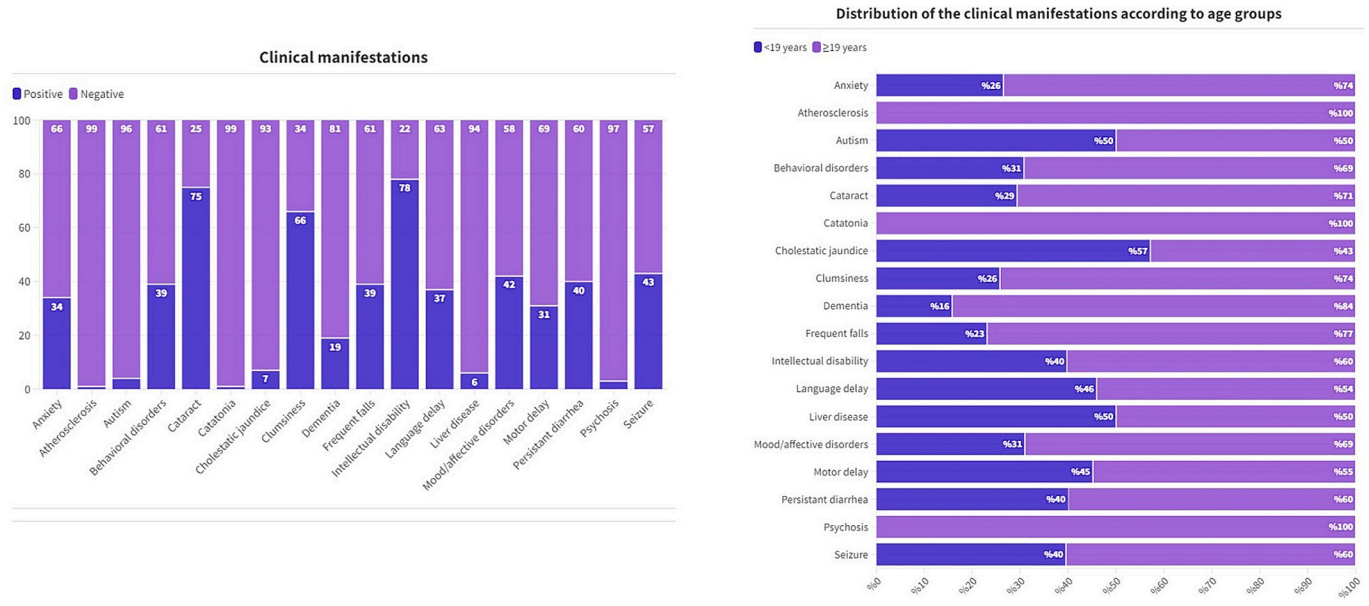


Fig. 3. Data on the distribution of clinical characteristics of the entire study cohort ($n = 100$ patients) and the percentage distribution of positive clinical findings in the pediatric ($n = 36$) and adult CTX patients ($n = 64$).

system involvement were found in 56% of patients (hyperreflexia in 55 and spasticity in 42 patients). In 18 of the patients, the findings were consistent with parkinsonism. Other signs of extrapyramidal system involvement such as dystonic movement disorders and myoclonus were found in 16 patients who did not have a full parkinsonian phenotype. Of the 47 patients in whom an initial EMG analysis was performed, 27 patients showed signs of peripheral neuropathy.

Three adult patients showed a milder phenotype without pyramidal, cerebellar and extrapyramidal involvement. Two of these patients had normal/slightly elevated plasma cholestanol levels. All three patients had xanthomas. One patient had cataract and one had psychiatric manifestations. One patient (P59–2) who had no sign of pyramidal, extrapyramidal and cerebellar involvement was excluded from this milder non-neurological phenotype group as she had a mild intellectual disability with an intelligence quotient score of 61. The data on the clinical, biochemical and genotypic characteristics of the patients with milder non-neurological phenotype are shown in Table 1.

Xanthomas were present in 60% of patients at the time of diagnosis. In 37 patients, the xanthomas were localized only in the Achilles tendons. One patient (P23–1) was diagnosed with CTX with a giant sacral xanthoma without neurologic involvement.

Four patients from three families were diagnosed with nephrolithiasis requiring medical treatment or surgical intervention (P68–2, P69–1, P70–1 and P70–3).

3.3. Neuroimaging data of the patients

Brain MRI data were available for 80 patients at the time of diagnosis. White matter changes were the most common radiologic sign and were observed in 43 (53%) patients. These white matter hyperintensities on FLAIR- and T2-weighted MR images were mainly localized in the subcortical and periventricular white matter of the frontal lobes and at the level of the centrum semiovale. Of the other MRI features, 25 showed cerebellar atrophy (31%), 24 showed cerebral atrophy (30%), 36 showed bilateral hyperintensity of the dentate nuclei (45%), and 23 showed cerebellar white matter involvement (28%). Magnetic resonance imaging of the spine could only be performed in 46 patients, and spinal involvement was found in 3 (6%) patients. Data on the distribution of neurologic imaging features are shown in Fig. 4.

3.4. Assessment of skeletal system involvement

Pes cavus deformity was present in 26% of all CTX patients. Data on BMD measurement were available for 52 patients at the time of diagnosis. 25 of these 52 patients were in the pediatric cohort. In 9 children with CTX, BMD *Z*-scores were below −2 SD. Three children who had a history of bone fracture were diagnosed with osteoporosis whereas the other six children were considered to have a low bone mass. 27 adult CTX patients had an initial BMD measurement. 11 of these 27 patients had BMD Z-scores were below −2 SD. Three children with a history of bone fracture had osteoporosis, whereas the other eight had low bone masses.

Table 1
Data on the clinical, biochemical and genotypic characteristics of the patients with milder non-neurological phenotype of CTX.

Patient ID	P23–1	P32–1	P33–2
Age of diagnosis(years)	65	40	26
Plasma cholestanol level (μg/ml)	33	7.5	5.7
Nucleotide change	c[1184 + 1G > A];[447-1G > A]	c.409C > T	c.409C > T
Initial symptom	Xanthoma	Xanthoma	Xanthoma
Mental retardation	−	−	−
Neurologic involvement	−	−	−
Psychiatric involvement	−	+	−
Cataract	+	−	−
Xanthomas	+	+	+

3.5. Evaluation of clinical phenotypes by age: Children versus adult patients

Thirty-six patients (36%) were diagnosed in childhood, 64 in adulthood. The mean age at diagnosis was 12.28 ± 4.50 years in children and 37.09 ± 9.17 years in adult patients. The delay in diagnosis was 6.71 ± 4.86 years in children and 24.96 ± 12.67 years in adult patients. Mean plasma cholestanol levels at diagnosis were not statistically different between the two groups ($p = 0.915$).

Both clinical and radiological neurologic findings dominated the clinical phenotype in adult patients compared to pediatric CTX patients. Statistical differences were significant for both pyramidal ($p < 0.001$) and extrapyramidal ($p < 0.001$) as well as cerebellar examinations ($p = 0.036$), with the difference being more pronounced for pyramidal and extrapyramidal abnormalities. In the cerebellar examination results, the frequency of intention tremor did not differ between the two groups ($p = 0.069$), demonstrating the high rate in pediatric CTX patients. The frequency of peripheral neuropathy detected by EMG analysis was not also statistically different between the two groups ($p = 0.234$).

Psychiatric manifestations were observed in both groups ($p = 0.396$). Anxiety ($p = 0.308$), mood/affective disorders ($p = 0.295$) and behavioral/personality disorders ($p = 0.414$) were part of the clinical phenotype in both children and adult patients.

In terms of extraneurologic findings, cataract ($p = 0.016$), xanthomas ($p < 0.001$) and changes in bone mineral density ($p = 0.03$) were also notable in the adult patients.

The clinical phenotype data and statistical analysis of manifestations by age group are shown in Table 2.

3.6. Biochemical and genotypic characteristics of the patients

Plasma cholestanol levels were measured in 93 patients. The mean plasma cholestanol level at the time of diagnosis before starting treatment was 27.81 ± 15.22 μg/ml. In seven patients, plasma cholestanol levels were found below 7 μg/ml. None of these patients had a history of additional medical treatment, particularly corticosteroids and cholesterol-lowering therapies, which can lead to a secondary decrease in plasma cholestanol levels.

In these 7 patients, *CYP27A1* gene analysis revealed 6 different variants. Two patients had the same variants (P24–1,P32–2). In this group, all reported variants were pathogenic or likely pathogenic according to the ACMG criteria. Normal plasma cholestanol levels were not associated with a mild phenotype, as six of the patients had an abnormal neurologic examination at the time of diagnosis. Only one patient (P33–2) presented mainly with xanthomas without neuropsychiatric findings and cataract. The data on the clinical, biochemical and molecular characteristics of the patients with normal cholestanol level are shown in Table 3.

Among the 100 patients from 72 families, 25 different variants were found. These 25 variants included nine missense, five frameshift, six splice-site, four nonsense, one intronic variant and one large deletion. The c.671_672del variant has not been described previously and this novel variant is defined as likely pathogenic according to the ACMG criteria. A total of 90 patients were homozygous for one variant, and 10 patients were compound heterozygous. The most frequent three variants were c.1476 + 2 T > C, c.808C > T and c.1263 + 4 A > T. No specific genotype–phenotype correlation was found. The data on the variants, variant type and ACMG classification are shown in Table 4.

4. Discussion

Cerebrotendinous xanthomatosis is a treatable neurometabolic disorder that is usually diagnosed in adulthood. Initiation of CDCA

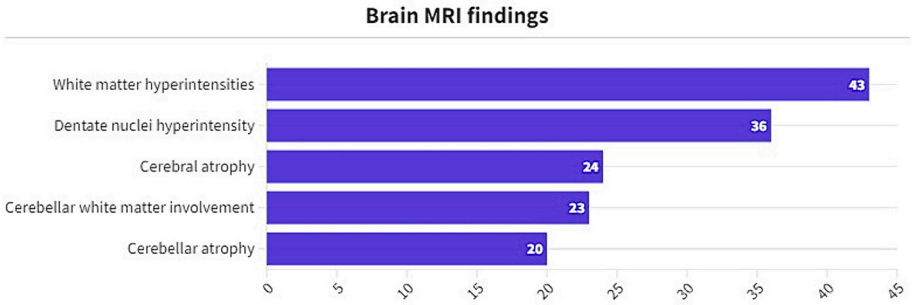


Fig. 4. Data on the distribution of brain imaging features of the patients (n = 80 patients).

Table 2
Comparison of the clinical phenotypes between pediatric and adult CTX patients.

	<19 years (n = 36)	≥19 years (n = 64)	p value*
Age at diagnosis (years)	12.28 ± 4.50	37.09 ± 9.17	**<0.001**
Age at onset (years)	5.57 ± 4.16	12.12 ± 11.06	**<0.001**
Diagnostic delay (years)	6.71 ± 4.86	24.96 ± 12.67	**<0.001**
Plasma cholestanol level (μg/ml)	27.80 ± 16.03	27.43 ± 14.73	0.915
Clinical Features			
Neuropsychiatric involvement			
Cerebellar signs	27/36	58/64	**0.036**
Ataxic gait	21/35	52/64	**0.03**
Dysarthria	8/35	46/64	**<0.001**
Nystagmus	1/35	14/63	**0.036**
Intention tremor	15/36	39/62	0.069
Pyramidal signs	9/36	47/63	**<0.001**
Spasticity	0/35	42/63	**<0.001**
Hyperreflexia	9/36	45/61	**<0.001**
Extrapyramidal signs	3/35	30/63	**<0.001**
Parkinsonism	0/35	18/63	**0.002**
Peripheral neuropathy	12/21	15/26	0.234
Brain MRI findings			
Cerebellar atrophy	2/32	23/48	**<0.001**
Cerebral atrophy	2/32	22/48	**<0.001**
Dentate nuclei hyperintensity	7/32	29/48	**0.001**
White matter hyperintensity	13/32	30/48	**0.036**
Cerebellar white matter involvement	2/32	21/48	**<0.001**
Psychiatric manifestations	21/35	40/64	0.396
Anxiety	9/32	25/60	0.308
Behavioral/personality disorders	12/34	26/62	0.414
Mood/affective disorders	13/34	29/57	0.295
Psychosis	0/35	3/64	0.176
Catatonia	0/35	1/64	0.310
Extraneurologic involvement			
Osteoporosis	9/25	11/27	**0.03**
Cataract	22/36	53/64	**0.016**
Xanthomas	7/36	53/64	**<0.001**

MRI, magnetic resonance imaging.
* Independent sample t-test for the comparison of quantitative data and Pearson's chi-square test for the comparison of categorical data; a P-value of <0.05 was considered statistically significant.

treatment prior to the onset of neurologic findings plays a key role in prognosis and treatment outcome, although a diagnostic delay of two decades has been reported. There are a few comprehensive studies of CTX patients in the literature, and these have generally been conducted in adult patients [4,5,18,27]. In this nationwide study, we present the phenotypes and genotypes of 100 patients with CTX from 72 families; to the best of our knowledge, this is the largest series ever reported. Data from 36 pediatric CTX patients were described to highlight the clinical phenotype in children and provide a comparison between pediatric and adult patients. The clinical presentation of seven patients with normal plasma cholestanol levels and three patients without neurologic involvement was also reported in contribution to the previous literature. 25 different variants were found; the c.671_672del variant has not been previously described and this novel variant is defined as likely pathogenic according to ACMG criteria.

In cohort studies, mainly of adult CTX patients, pyramidal and cerebellar symptoms have been reported to dominate the neurologic spectrum of the disease [4,5,18,27–29]. Extrapyramidal system involvement has been emphasized as a less common component of the neurologic phenotype. Parkinsonism is the most commonly reported extrapyramidal sign, but findings other than parkinsonian symptoms, such as dystonic movement disorders, myoclonus, and postural tremor, have also been reported [28–31]. Myoclonus was cited as one of the most common but underdiagnosed extrapyramidal finding of CTX and the need for electrophysiological examination to differentiate myoclonus from intention tremor or action tremor has also been pointed out [32]. In spinal xanthomatosis, which is considered a clinical and radiologic subtype of CTX, the initial neurologic findings reported were spastic paraparesis, hyperreflexia, a positive Babinski sign, and proprioceptive symptoms; cerebellar signs and cognitive impairment were less common [33]. Although the incidence of peripheral neuropathy has been reported to be very close to pyramidal and cerebellar involvement, it was generally detected by EMG analysis, as patients did not develop symptoms until later stages [4]. Epilepsy and intellectual disability have also been reported as frequent and earlier findings of neurologic involvement [4,5]. In our study, intellectual disability (78%) was one of the most common clinical manifestations of the disease. The neurologic findings were consistent with the literature, with a predominance of cerebellar involvement (84%) followed by pyramidal involvement (56%). Patients also presented with both parkinsonism (18%) and nonparkinsonian extrapyramidal involvement (16%). Peripheral neuropathy was a common finding in our cohort.

Multiple psychiatric involvement at different ages has been mentioned as an important clinical feature. In a Dutch cohort, autism was described in 10 of the 77 patients and highlighted as an early and probably underestimated common feature of the disease [34]. Psychiatric manifestations in addition to autism included behavioral/personality disorders, mood/affective disorders, psychosis, catatonia, anxiety, and dementia. Fraidakis et al. observed a bimodal/bitemporal pattern in the manifestations of CTX. In the later stages of the disease, affected individuals may present with dementia and a spectrum of neuropsychiatric symptoms that includes behavioral and personality disorders, affective and mood disorders, and psychotic disorders. In contrast, in the earlier stages of the disease, particularly in childhood and adolescence, psychiatric manifestations are characterized by learning difficulties or intellectual disability [35]. In our cohort, four patients showed signs consistent with autism. Psychiatric manifestations other than autism were also highlighted as a dominant feature, as 61% of patients had at least one psychiatric finding.

The most common systemic findings reported were cataracts, tendon xanthomas and persistent diarrhea [4,5,18,27]. Since cataract is

Table 3
Data on the clinical, biochemical and genotypic characteristics of the patients with normal cholestanol level.

Patient ID	P2-2	P3-1	P24-1	P33-2	P36-1	P43-1	P59-1
Plasma cholestanol level (μg/ml)	4.37	3.13	5.45	5.77	6.07	3.76	6.78
Age of diagnosis(years)	17	44	32	26	39	31	53
Nucleotide change	c.1435C > T	c.1538G > T	c.409C > T	c.409C > T	c.646G > C	c.1476 + 2 T > C	c.[1_5del];[1184 + 1G > A]
Mental retardation	+	−	−	−	−	+	−
Neurologic involvement	+	+	+	−	−	+	+
Psychiatric involvement	−	−	−	−	+	−	−
Cataract	+	−	−	−	−	+	+
Xanthomas	−	+	−	+	+	+	+

Table 4
Data on the CYP27A1 gene analysis: variants, mutation type and ACMG classification.

Nucleotide change*	Protein change	Mutation type	ACMG classification	Reference
c.808C > T	p.(Arg270Ter)	Nonsense	Pathogenic	Ahmed et al. [50]
c.1435C > T	p.(Arg479Cys)	Missense	Pathogenic	Cali et al. [51]
c.1538G > T	p.(Arg513Leu)	Missense	Likely pathogenic	Chen et al. [52]
c.409C > T	p.(Arg137Trp)	Missense	Pathogenic	Nakashima et al. [53]
c.671_672del	p.(Lys224ThrfsTer63)	Frameshift	Likely pathogenic	**novel variant**
c.1476 + 2 T > C		Splice-site	Pathogenic	Lagarde et al. [31]
c.608C > A	p.(Ser203Ter)	Nonsense	Pathogenic	Kısa et al. [6]
c.1263 + 4A > T		Splice-site	Uncertain significance	Kısa et al. [6]
c.1333C > T	p.(Gln445Ter)	Nonsense	Pathogenic	Chang et al. [54]
c.646G > C	p.(Ala216Pro)	Missense	Pathogenic	Garuti et al. [55]
c.1151C > T	p.(Pro384Leu)	Missense	Benign	Verrips et al. [5]
c.774delC	p.(Lys259SerfsTer27)	Frameshift	Likely pathogenic	Yunisova et al. [28]
c.447-1G > A		Splice-site	Pathogenic	Garuti et al. [56]
c.1028C > G	p.(Thr343Arg)	Missense	Likely pathogenic	Pilo-de-la Fuente et al. [18]
c.1184 + 1G > A		Splice-site	Pathogenic	Garuti et al. [56]
c.1183C > T	p.(Arg395Cys)	Missense	Pathogenic	Cali et al. [51]
exon6del		Large deletion	Pathogenic	Lee et al. [57]
c.1016C > T	p.(Thr339Met)	Missense	Pathogenic	Reshef et al. [58]
c.508_509ins	p.(Glu170ValfsTer16)	Frameshift	Pathogenic	Baltacı et al. [59]
c.850 A > T	p.(Lys284Ter)	Nonsense	Pathogenic	Meiner et al. [60]
c.1_5del	p.(Met1?)	Frameshift	Likely pathogenic	Yunisova et al. [28]
c.256-1G > C		Splice-site	Likely pathogenic	Zubarioglu et al. [7]
c.446 + 1G > A		Splice-site	Pathogenic	Verrips et al. [5]
c.1571 T > G	p.(Leu524Arg)	Missense	Likely pathogenic	Mutlu et al. [61]
c.11_20del	p.(Leu4ArgfsTer3)	Frameshift	Likely pathogenic	Lee et al. [57]

* transcript: NM_000784.

considered an early and common sign of the disease, prospective screening studies have been conducted to identify the CTX patients before the appearance of neurologic findings. In these studies, CTX prevalence in the juvenile idiopathic cataract group was reported to be between 0.99 and 3.3% [36–38], which is significantly higher than the estimated CTX prevalence in the general population. Diarrhea has been reported as an early and frequent sign of CTX. However, the defecation pattern has no specific features and generally receives less attention until neuropsychiatric findings emerge [39]. An increased risk of fractures due to low bone mass and osteoporosis in CTX was first described by Berginer et al. [40]. Following the description of skeletal involvement in CTX, numerous studies have documented the correlation between bone metabolism and CTX [38–40]. Cataract (75%), xanthomas (60%) and persistent diarrhea (40%) were also common systemic findings in our cohort. Osteoporosis was assessed by BMD z-scores in 52 patients, with 6 patients found to have osteoporosis (3 children and 3 adults) and 14 patients with low bone mass (6 children and 8 adults). In our study, four patients were found to have nephrolithiasis requiring treatment. In the medical literature, kidney stones have been mentioned in individual case reports, but a clear relationship between kidney stones and CTX could not be established [41,42].

Considering the age of onset and diagnosis in CTX patients, it is clear that there is a diagnostic delay of at least two decades [4,5,18,27,43]. Data on the natural history of adult CTX patients suggested a clinical phenotype consisting of neuropsychiatric findings in adults, whereas extraneurologic findings were common in children. Mignarri et al. documented that CTX manifests with chronic diarrhea and intellectual disability in childhood, whereas cataracts and epilepsy were more prominent in adolescence. Neuropsychiatric changes typically appeared after the second decade of life. Osteoporosis and parkinsonism had been reported as later findings of the disease [4]. The results of a large Dutch sample cohort were also consistent with Mignarri's data [5]. In a case series of five CTX patients and a literature review, Wong et al. classified the neurologic abnormalities of CTX according to the time of onset of each symptom using a cumulative incidence function analysis. Ataxic gait disturbances, corticospinal tract abnormalities, seizures, psychiatric manifestations and speech changes were observed throughout the life course, while parkinsonism and sensory loss tended to occur relatively late in the course of the disease [8]. However, the evaluation of pediatric CTX studies highlighted a neuropsychiatric involvement in CTX that can be observed early in the course of the disease, even in early childhood. In a pediatric CTX study conducted with six children, none of the patients showed pyramidal and extrapyramidal signs, but all showed at least one cerebellar sign, in particular intention tremor. Peripheral neuropathy was observed in five patients, and psychiatric manifestations in three patients. Severe osteoporosis with a history of fractures due to minor trauma was noted in two patients [7]. The results of another pediatric CTX study in Turkey were consistent with the findings of Zubarioglu et al. as six out of seven pediatric patients had ataxia and four had polyneuropathy. Two patients had early osteoporosis, one of whom had a history of fractures [6]. In our study, 36 patients were diagnosed in childhood, which is considered as a contribution to the current literature. Pyramidal and extrapyramidal findings were significantly more frequently observed in adults. This difference was also present in the cerebellar findings, but less pronounced. When cerebellar findings were considered separately, the frequency of intention tremor did not differ between the two groups, demonstrating the high rate in pediatric patients. The frequency of peripheral neuropathy and psychiatric

manifestations also did not differ statistically between the two groups. According to these results, abnormal cerebellar findings, especially intention tremor, peripheral neuropathy, and psychiatric findings associated with intellectual disability, have been suggested as important features for early diagnosis of CTX in children.

Cerebrotendinous xanthomatosis is considered a clinically heterogeneous disease, and a milder phenotype without overt neuropsychiatric involvement has also been reported in the literature. In a Dutch series, 19 patients were reported in which cataract (21%) and xanthomas (84%) were the only or dominant clinical manifestations of the disease. Hyperlipidemia was a common concomitant laboratory finding, and plasma cholestanol levels were also elevated [9]. Tama Viteri et al. also reported on a 64-year-old female patient with a history of progressively enlarging xanthomatous soft tissue tumors. Cutaneous, subcutaneous and tendinous lesions with a pseudotumoral nodular appearance in the presence of hyperlipidemia led to the misdiagnosis of familial hypercholesterolemia and sitosterolemia, and CTX was finally diagnosed after molecular analysis of *CYP27A1* [10]. The likelihood of neuropsychiatric involvement in these patients is still unclear. In the case report by Tama Viteri et al., no neuropsychiatric findings were observed either at diagnosis or during the follow-up period. However, Stelten et al. reported a worsening of the Expanded Disability Status Scale and psychiatric signs during the follow-up period in some patients [9,10]. The data from our study added three new patients to the mild phenotype patient group described in the previous literature. Two of our patients had normal/slightly elevated plasma cholestanol levels, and xanthomas were a common finding consistent with the literature. Due to the psychiatric involvement of two patients with a milder phenotype, treatment and close monitoring of these patients was recommended.

Measurement of the plasma cholestanol level in the presence of a total Mignarri score $\geq$ 100 was recommended as the first step in the diagnostic approach of CTX [4]. In the expert opinion on CTX, measurement of serum cholestanol was accepted as the reliable diagnostic marker of choice, and in ranking the importance of tests to confirm a CTX diagnosis, panelists agreed that *CYP27A1* gene sequencing was the most important, followed by determination of serum cholestanol levels [12]. However, neurologically impaired and molecularly confirmed CTX patients with normal plasma cholestanol levels have also been reported [44]. On the other hand, liver diseases, familial hypercholesterolemia and sitosterolemia have been reported to be associated with elevated plasma cholestanol levels [45,46]. These findings suggest that biochemical markers other than cholestanol are needed. Comprehensive analysis of lipid metabolites in CTX patients revealed an increased accumulation of intermediates of the 7α- and 25-hydroxylation pathways, whereas 27-hydroxylated and carboxylated cholesterol products were largely absent [2,47]. As a result, the increase in 7α,12α-diHCO and the absence of 27-hydroxycholesterol (27-HC) or their ratio have been recommended as primary and reliable biochemical diagnostic biomarkers in CTX [2,48]. In our study, seven patients had plasma cholestanol levels below 7 μg/ml. Low plasma cholestanol levels have been reported as a consequence of treatment with corticosteroids and ezetimibe in the medical literature [44]. In our study, the medical records of these seven patients were reviewed in detail and no history of interfering medication was found. Normal plasma cholestanol levels were not associated with a mild phenotype. Based on these results, further biochemical testing and molecular analysis of the *CYP27A1* gene was recommended when suggestive clinical findings are present despite a normal plasma cholestanol level.

To date, 145 variants have been reported in the *CYP27A1* gene [49]. A definitive correlation between genotype and phenotype has not been established. In our study, 25 different variants were found among the 100 patients from 72 families. The c.671_672del variant has not been described previously and this novel variant is defined as likely pathogenic according to the ACMG criteria. Analysis of the *CYP27A1* gene in one patient (P14–1) revealed the variant c.1151C > T (p.Pro384Leu) in a homozygous state, which was defined as benign according to the ACMG criteria. This variant has been reported to have an allele frequency of $\geq$1% in European, Latin American and Asian populations and has been hypothesized to cause a classic CTX presentation when present in trans with a null allele [16]. However, the same variant has been previously described as a cause of a classic CTX phenotype and has been considered as pathogenic in the literature [5]. Our patient had cataract, intellectual disability, developmental delay, persistent diarrhea, cerebellar findings and a high plasma cholestanol level. The CYP27A1 gene analysis was re-evaluated and no other variant could be identified. Depending on the clinical and biochemical findings consistent with CTX, the c.1151C > T variant was also considered responsible for the disease in these patients, similar to the observation of Verrips et al.

Our study had some limitations. The most important limitation was that no follow-up data were presented. In Turkey, CDCA treatment is routinely recommended for all CTX patients after genetic confirmation of the diagnosis. The drug is also accessible in Turkey. All authors agreed on the importance of a longitudinal study reporting the CDCA treatment results, but the follow-up data were excluded to ensure the confidence and reliability of the study, as there were no homogeneous data regarding treatment dose and follow-up frequency in a large number of patients from different centers. Authors also agreed on the importance of CDCA treatment for improved prognosis and, although no treatment follow-up data is provided here, believe the data presented illustrates the expanding phenotypic spectrum of disease recognized for CTX and could be useful for early diagnosis. The second limitation was the fact that MRI data and nerve conduction studies were not available for the entire study group.

5. Conclusion

In conclusion, this first nationwide comprehensive Turkish series of CTX patients emphasizes that the true prevalence of the disease is probably underestimated and that it has a broad spectrum of clinical phenotypes even without neurologic impairment. In children, abnormal cerebellar findings, especially intention tremor, peripheral neuropathy, and psychiatric findings associated with intellectual disability, have been suggested as important features to avoid diagnostic delay. Cholestanol is a reliable diagnostic option. However, since severe neurologic involvement can occur despite normal plasma cholestanol levels, molecular analysis should also be performed if there is clinical suspicion, even though the cholestanol level is normal.

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Ethical approval

This study was designed in accordance with the current revision of the Helsinki declaration and was approved by the local Ethical Committee of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty (E-83045809-604.01.01-873,441).

Informed consent

Informed consent was obtained from all patients for being included in the study.

CRediT authorship contribution statement

Tanyel Zubarioglu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ertuğrul Kıykım:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Çiğdem Aktuğlu-Zeybek:** Writing – review & editing, Writing – original

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Declaration of Competing Interest

Authors state no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2024.108493.

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