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SHORT COMMUNICATION

A novel frameshift mutation in the sterol 27-hydroxylase gene in an Egyptian family with cerebrotendinous xanthomatosis without cataract

Mohamed S. Abdel-Hamid[1] · **Mahmoud Y. Issa**[2] · **Ghada A. Otaify**[2] · **Maha S. Zaki**[2]

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Abstract Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive lipid storage disorder caused by deficiency of the mitochondrial cytochrome P450 sterol 27-hydroxylase enzyme encoded by *CYP27A1* gene. CTX is characterized by tendon xanthomas, juvenile cataracts and multiple progressive neurological symptoms. Here we report on the clinical and molecular findings of a 35-years old Egyptian patient with CTX without cataract. Parents were first cousins with family history of two deceased sibs with mild impaired cognitive functions and epilepsy without appearance of tendon xanthomas. Our proband had learning disabilities and developed seizures at 9 years old. Tendon xanthomata appeared at the age of 16 and his neurological symptoms remained stationary till 28 years followed by progressive cerebello-pyramidal signs, dementia and psychiatric disturbance. Cataract was not evident in our patient. Brain MRI showed the characteristic focal lesions appeared as xanthomas in cerebellum and occipital horns of lateral ventricles. Molecular study identified a novel homozygous frameshift mutation in *CYP27A1* gene, c.1169delT (p.K391Rfs*17). Our study emphasizes the important role of early genetic testing in prevention of morbidity and mortality of the disease and proper counseling. Moreover, it shows that the absence of cataract should not rule out the diagnosis of CTX.

Keywords Cerebrotendinous xanthomatosis · *CYP27A1* gene- cerebellar manifestations · Xanthomas · Cataract · Cholestanol

Introduction

Cerebrotendinous xanthomatosis (CTX, OMIM #213700) is a rare autosomal recessive progressive lipid-storage disorder due to deficiency of sterol 27-hydroxylase encoded by *CYP27A1* gene. The main criteria of the disease are juvenile bilateral cataract, tendon xanthomas, chronic diarrhea and broad neurological symptoms which appears insidiously and become evident by the second decade of life. However, there is a clear variability in clinical presentation, age at onset, specific symptoms and severity of the disease even among same pedigree (Verrips et al. 2000; Pilo-de-la-Fuente et al. 2011; Mignarri et al. 2014).

Early neurological symptoms include psychomotor retardation, cognitive impairment, learning difficulties, and epilepsy in 50% of patients (Mignarri et al. 2014; Nie et al. 2014). Later, patients develop progressive spastic paraplegia due to pyramidal tract affections, progressive cerebellar ataxia, nystagmus, dysarthria, peripheral nerve affection, intellectual deterioration, dementia, psychosis and rarely movement disorder as parkinsonism, palatal myoclonus and blepharospasm (Fraidakis 2013; Lagarde et al. 2012). CTX is a potentially treatable metabolic disease, and early diagnosis is crucial to prevent neurological damage and deterioration. Diagnosis is confirmed by assaying elevation of cholestanol and cholesterol precursors levels in the plasma or cerebrospinal fluid (Björkhem 2013) and by genetic testing of sterol 27-hydroxylase gene *(CYP27A1)* (Sugama et al. 2001).

Herein, we report the first Egyptian family with classic manifestations of CTX but lacking cataract, one of the

✉ Maha S. Zaki
dr_mahazaki@yahoo.com

[1] Medical Molecular Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Giza, Cairo, Egypt

[2] Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Tahrir street, Dokki, Giza, Cairo 12311, Egypt

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cardinal features of the disease, and confirmed by identifying a novel mutation in *CYP27A1* gene.

Clinical report

A 35 years old male patient presented with loss of acquired skills started at the age of 28 years old. He was the second offspring to healthy consanguineous parents with history of deceased female and male sibs (IV-3-4) (Fig. 1a). Both had mild mental retardation, seizures around the age of 8 and disturbed mood with aggressive behavior near puberty. The female had disturbed gait at 18 years old and died with undiagnosed lung problems at the age of 20 while the male died suddenly at 15 years old. Both lacked tendon xanthomatosis and cataract and were not investigated.

Our proband had normal developmental milestones but with notable learning disability. At the age of 9, he developed generalized tonic-clonic seizures, fairly controlled on valproate and carbamazepime. The first tendon xanthoma appeared at the age of 16 in the left ankle that was excised. Its pathology verified yellowish white mass with excessive deposition of cholesterol crystals with many xanthoma cells and multinucleated giant cell microscopically suspected cholesterol granulomata/ xanthogranulomatous reaction. Subsequently, several tendon xanthomas appeared and some recurred after excision. Progressive deterioration started at the age of 28 with recurrent falling, uncoordinated gait and became confined to wheel chair at 32 years with regression of speech and dementia. Psychological disturbance manifested as bouts of unexplained anger and crying was experienced lately. No history of cataract recorded.

On examination, patient was only able to sit and responded to command without verbal interaction. He showed behavioral disturbance with bouts of excessive weeping. General examination was irrelevant except for multiple tendon xanthomatosis in elbows, knees and ankles (Fig. 1b). No xanthelesma was detected.

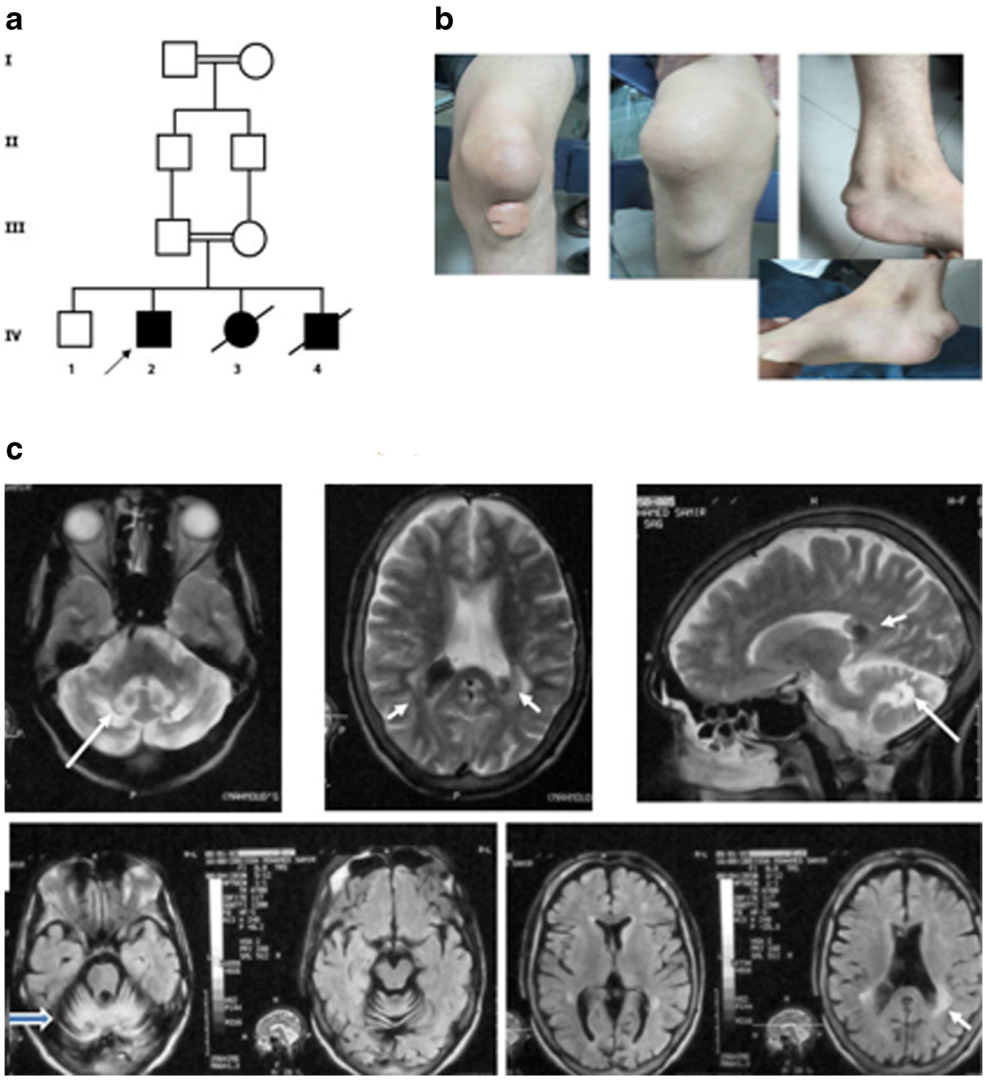

Fig. 1 **a** Pedigree of the studied family. **b** Lower limbs of our proband showing bilateral patellar and infra-patellar tendon xanthomas and bilateral tendo-achilles xanthomas. **c** MRI brain; T2W (*upper row*) and T2W-FLAIR (*lower row*) showing bilateral high signal intensity of the cerebellum white matter and low signal intensity of the deep cerebellar nuclei (*white long arrow*), cerebellar atrophy (*thick arrow*), area of low signal intensity surrounded by high signal intensity of the white matter around the occipital horns which is more evident in right side (*white short arrows*)

Neurological assessment showed lateral nystagmus, infrequent drooling, intention tremors and incoordination. His muscle power in upper limbs was grade 4 while the lower limbs showed more affection of antigravity muscles (grade 3). He had hypotonia, brisk reflexes, ankle clonus, positive Babiniski sign, pes cavus, normal superficial sensation and average sphincteric control. Investigations revealed normal abdominal sonar, echocardiography, EMG and NCV. Ophthalmological assessment using fundoscope and ultrasound showed clear lens, normal choroid, retina and optic nerve. Bilateral fronto-temporal epileptogenic dysfunction was in EEG and brain MRI showed mild cortical and cerebellar atrophy, high signal of the white matter especially around the occipital horn, focal high signal lesions at T2 bilateral within the cerebellum and deep in cerebrum, and low signal focal lesions at the vicinity of cerebellar nuclei and the occipital horns of lateral ventricles more pronounced in right side (Fig. 1c). All blood chemistry parameters were normal. Unfortunately, we couldn't perform screening with serum cholestanol as it wasn't available in Egypt, so we proceeded to molecular testing.

Beginning of treatment with chenodeoxycholic acid (CDCA) of 250 mg three times for 3 months found to be highly effective in regression of psychotic manifestations and stabilization of his mood, nevertheless no regression of other neurological manifestations was noted.

Mutation analysis of *CYP27A1* gene

Genomic DNA was extracted from peripheral blood lymphocytes of the patient, his parents and available family members after having a signed informed consent according the guidelines of the Research Ethical Committee of the NRC. The *CYP27A1* gene was amplified using specific primers designed using EXON PRIMER and directly sequenced in both directions in our patient. Primers are available upon request. The sequence data of *CYP27A1*gene was compared with the reference genomic and cDNA sequence of the gene (NM_000784.3). Sequence analysis identified a novel homozygous 1-bp deletion in exon 6 of the gene, c.1169delT (p.K391Rfs*17) (Fig. 2a). The mutation was found in the heterozygous state in both parents as well as the healthy sibling. The p.K391Rfs*17 mutation was not found in the public databases like dbSNP, 1000G and ExAC. Further, it was not detected in 100 normal control of Egyptian origin by PCR and restriction fragment length polymorphism analysis (Fig. 2b).

Discussion

Cerebrotendinous xanthomatosis is a rare progressive neurological disease characterized by accumulation of cholesterol and cholestanol in brain, tendons and other system involvement. Diagnosis of CTX is usually delayed due to insidious onset, progressive course, variability in clinical manifestations, incomplete phenotype till advanced age and rarity of the condition in addition to under or misdiagnosis (Nie et al. 2014; Degos et al. 2016).

Diagnosis of the disease in our patient based mainly on clinical and neuro-radiological examinations followed by genetic testing of the *CYP27A1* gene. Molecular studies of the *CYP27A1* gene identified a novel homozygous single nucleotide deletion mutation in exon 6 of the gene. This deletion induces an amino acid change and early protein truncation and is predicted to result in a nonsense-mediated mRNA

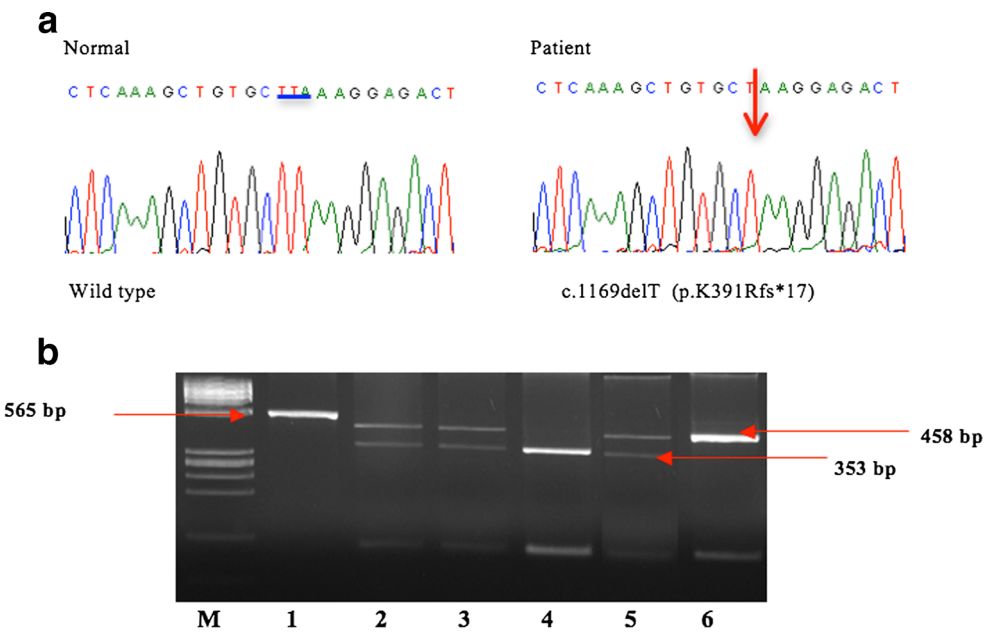

Fig. 2 **a** Portion of the sequencing electrophoregram showing the site of mutation. **b** 3% agarose gel showing PCR-RFLP analysis of the mutation using DdeI restriction enzyme (Fermentas, Germany). Lane 1: Undigested PCR product (565 bp). Lanes 2,3&5: Father, mother and healthy sibling, heterozygous for the mutation (458, 353, 107, 105 bp). Lane 4: The male patient, homozygous for the mutation (353, 107, 105 bp). Lane 6: Normal control subject with the wild type sequence of exon 6 (458, 107 bp). Lane M: PhiX DNA ladder (Finzyme, Finland)

decay. This mutation was not found in the dbSNP, 1000G or ExAC databases and was predicted to be disease causing by various bioinformatics tools. Furthermore, it was not detected in 200 normal chromosomes of Egyptian origin excluding the possibility of being a rare variant.

To date, more than 57 distinct disease-causing mutations in the *CYP27A1* gene have been reported with no phenotype-genotype correlations and none of these mutations are preferentially associated with specific symptoms (Pilo-de-la-Fuente et al. 2011; Ragno et al. 2015**;** Di Taranto et al. 2016). Moreover, Appaduri et al. (2015) identified 29 genetic variants with strong bioinformatics support of pathogenicity through analyzing 60,000 unrelated adults from global population. The majority of mutations are missense (around 50%) and are clustered in the region spanning exons 6–8 which is in accordance with the location of mutation found in our patient. Other types of reported *CYP27A1* mutation include nonsense (22%), splice site (20%) and small deletions and insertions (18%) (Ragno et al. 2015**;** Di Taranto et al. 2016).

Although bilateral juvenile cataract is considered one of the major manifestations of the disease, it wasn't recorded in our patient. Nevertheless, in literature among three diverse large cohort groups in 2000, 2011 and 2014, cataract was in 90%, 92% and 88% of patients **(**Verrips et al. 2000**;** Pilo-de-la-Fuente et al. 2011; Mignarri et al. 2014**)**. Further, Guyant-Maréchal et al. (2005) described a 53 years-old man with late presentation at the age 44 with dementia, but without cataract or ataxia and and Di Taranto et al. (2016) reported a patient without cataract or any neuropsychological manifestations till the age of 32 years carrying a homozygous missense mutation (p.R479G). Authors postulated a possible phenotype-genotype correlation of a milder phenotype related to this particular mutation supported by the report of Guyant-Maréchal et al. (2005) that showed the same variant as a compound heterozygous with another missense variant (p.T339 M). However, relying this milder phenotype to the type of mutation alone is not strong enough specially that many missense mutations in the *CYP27A1* gene have been associated with severe phenotype of CTX (Nie et al. 2014**)**. Involvement of genetic modifiers or environmental interactions in this scenario cannot be excluded. Therefore, with this additional report we point that cataract might not be a constant finding of the disease and more likely not related to specific mutation(s).

We expect that the two sibs of our patient with behavioral abnormalities and seizures suffered from the same genetic condition and succumbed before twenties from pulmonary problem and sudden death possibly cardiac cause, respectively which are the main systemic involvements of the disease. Both had no tendon xanthomata. Similarly, no early onset cataract was recorded. Tendon xanthomas were missed in more than 50% of cases in 2 cohort studies at time of diagnosis (Verrips et al. 2000**;** Degos et al. 2016**)**. This emphasizes that the absence of xanthomas shouldn't rule out or delay diagnosis of CTX.

Neonatal cholestasis and chronic diarrhea from childhood could be the earliest presentation of CTX and are considered among the cardinal features, however our patient and his sibs didn't encounter any of these symptoms. Two reports have previously described late presentation of chronic diarrhea including an Italian patient at the age of 24 years (Ragno et al. 2015) and a Colombian patient after age of 50 years (Giraldo-Chica et al. 2015).

In several reports, it has been shown that neurological and non-neurological manifestations can respond well to CDCA through decreasing plasma cholestenol level especially if initiated early (Bonnot et al. 2010; Nie et al. 2014; Degos et al. 2016). Our patient showed good response and stabilization of his psychiatric status after 3 months of treatment.

In conclusion, this study emphasizes that patients with unexplained learning difficulties, mild cognitive impairment, personality changes and seizures should be screened for *CYP27A1* mutations for early diagnosis and treatment. Diagnosis of presymptomatic family members is also important to minimize the morbidity and mortality and improve the prognosis of this genetic disease.

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