Cerebrotendinous xanthomatosis: recurrence of the CYP27A1 mutation p.Arg479Cys in Sardinia

UNIVERSITÀ DEGLI STUDI DI TORINO

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Introduction

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive disorder, due to mutations of the *CYP27A1* gene, leading to cholestanol accumulation because of the sterol-27-hydroxylase enzyme deficiency [1].
Treatment with chenodeoxycholic acid (CDCA) normalizes cholestanol and improves neurophysiological findings.
Several mutations of the *CYP27A1* gene are described in different ethnic groups [1], without genotype–phenotype correlations. We describe two unrelated Sardinian families sharing the same *CYP27A1* mutation.

Family 1
A 47 year-old-man, born from consanguineous parents from Olbia (northeastern Sardinia), showed cataracts at age 21, progressive walking difficulty since age 24 and severe bipolar disorder at 39. At age 43 tendon xantomas were identified.

Neurological examination revealed pareto-ataxic gait, hyperactive tendon reflexes, extensor plantar responses, asymmetrical accommodation reflex, mild cognitive impairment, defective attention and short-term memory deficit (Mini Mental Status Examination (MMSE): 21/30). Electromyography (EMG) revealed sensorimotor axonal polyneuropathy.
Total cholesterol was 232 mg/dl.
Electrocardiogram (ECG) showed signs of a previous myocardial infarction. Echocardiogram, brain and spinal cord Magnetic Resonance (MRI) were normal.
The patient never received CDCA treatment.
Cataracts and tendon xanthomas were also referred in his three brothers, who refused the genetic testing.

Family 2
Parents are first cousins from the Carbonia-Iglesias area (southwestern Sardinia).
The proband, male, was evaluated at 45 years because of progressive walking difficulty. A diagnosis of spastic paraplegia was hypothesized but linkage analysis pointed to *CYP27A1* locus. Neurological examination showed paretic gait, hyperactive tendon reflexes, extensor plantar responses, tendon xanthomas, buccal dyskinesia and moderate mental retardation. EMG demonstrated a mild sensorimotor polyneuropathy. Brain MRI showed multiple areas of white matter alterations.
Serum cholestanol was 64.9 mg/l (nv<10) and cholesterol 254 mg/dl.
Since age 53 he assumes CDCA 750 mg/die, with xantomatas reduction but no neurological improvement.

The sister (46 years) reported two elbow neoformations removal (histology not available), progressive walking difficulties since age 34 and cataracts at 44 years. Neurological examination showed paretic gait, extensor plantar responses, long term attention deficit and a mild cognitive impairment (MMSE 20/30). Brain MRI was normal; spinal MRI showed a non-homogeneous cervical signal. ECG revealed an incomplete right bundle branch block. Serum cholestanol (28.3 mg/l) and total cholesterol (238 mg/dl) were increased.
Since age 45 she assumes CDCA 750 mg/die, with symptomatology stabilization.

Molecular analysis

Exons 1-9 and the promoter of the *CYP27A1* gene were sequenced (protocol available upon request). Both families share the same homozygous mutation c.1435C>T, p.Arg479Cys, previously known as p.Arg446Cys [2], described as pathogenic in dbSNP (rs72551322) and reported only among European-American (MAF: 0.0001) in the Exome Variant Server (http://evs.gs.washington.edu/EVS/ [01,2014 accessed]).

Two healthy relatives in family 2 (a 39 year-old niece and a 37 year-old nephew) carried a variant haplotype with c.888A>G, p.Gln296Gln (rs61733619) and c.1471G>T, p.Ala491Ser (rs72551323) variants, recorded in dbSNP as variants of unknown significance. The first has a MAF of 0,0233 among European-American. *In silico* splicing prediction didn't show alterations (GeneSplicer: www.cbcb.umd.edu/software/GeneSplicer/gene_spl.shtml; MaxEntScan: http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html; NNSplice: www.fruitfly.org/seq_tools/splice.html). The p.Ala491Ser (MAF: 0,0019 among European-American) is predicted to be benign (SIFT: http://sift.jcvi.org/; PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/; Mutation Taster: www.mutationtaster.org).
The benign significance is also supported by the healthy status of the niece, who resulted to be a compound heterozygote with the p.Arg479Cys and the variant haplotype. Indeed, both she and her brother, who carries only the variant haplotype, have normal cholestanol (4.1 µg/l and 3.8 µg/l respectively).

Discussion

The phenotype of our patients is characteristic of CTX.
The frequency of this disorder varies among populations and is higher in inbred ethnic groups [3]. In geographically isolated populations, such as Sardinia, the finding of founder mutations is expected [4].
The p.Arg479Cys mutation was described either in homozygotes or compound heterozygotes [2, 5]. The affected Arginine is located next to Cysteine476 that binds the heme cofactor, thus it is predicted to alter enzyme activity. Transfection of mutant cDNAs into cells results in the synthesis of sterol 27-hydroxylase protein with greatly diminished enzyme activity [2].

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