

Title of the article: Cavitating leukoencephalopathy with posterior predominance caused by a deletion in the *APOPT1* gene in an Indian boy

Running title: *APOPT1* gene pathogenic variant associated cavitating leukoencephalopathy

Category: Case report

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Cavitating leukoencephalopathy with posterior predominance caused by a deletion in the *APOPT1* gene in an Indian boy

Abstract

A five-year-old Indian boy presented with subacute onset regression of milestones associated with seizures and spasticity. The symptoms started after an attack of measles. The MRI of the brain showed cavitating leukodystrophy with posterior predominance. Molecular analysis of the *APOPT1* gene, a recently described gene associated with mitochondrial leukodystrophy, showed the patient to be homozygous for a 12.82 kilobase deletion, including coding exon 3. Deletion of exon 3 produces a frameshift, predicting the translation of a truncated protein (p.Glu121Valfs*4). The patient was started on mitochondrial cocktail regimen of thiamine, riboflavin, Co-enzyme Q and carnitine. Although he initially showed some improvement, he succumbed six months after the onset of his illness.

Key words: inborn error of metabolism; seizures; mitochondrial disorder; MRI; leukodystrophy

Introduction

Recently pathogenic variants (earlier called mutations) in the *APOPT1* (Apoptogenic 1) gene have been reported to cause infantile or childhood onset mitochondrial disease with clinical features varying from acute neuroregression in late infancy to chronic neurodegeneration presenting in adolescence.(1) This disease is characterized by deficiency of cytochrome c oxidase (COX) and a peculiar neuroimaging appearance of cavitating leukodystrophy with posterior cerebral predominance. Only six cases have been reported before, in an earlier study.(1) We report an Indian boy who presented with subacute neuroregression after an attack of measles.

Case Report

A five year old, previously normal boy was brought to us with complaints of regression of milestones since the last 3 months. He was apparently well 3 months back, when he developed measles, from which he recovered. Ten days later, he started having right sided focal seizures, multiple times a day. Within the next 3 weeks, he stopped walking, and sitting. Over the next 2 months, he stopped talking, recognizing his parents and interacting. He also developed severe stiffness in all the four limbs. He also lost the ability to see and hear. He was the third child of third degree consanguineous parents. The family history was not significant.

Examination revealed a bed bound child in vegetative state. The vitals were stable and the anthropometric parameters were appropriate for age. The general physical and systemic examinations were normal. The cranial nerve examination including the ocular fundi was

normal. Motor examination revealed decorticate posturing and hypertonia in all four limbs, with brisk deep tendon reflexes. The plantar responses were extensor bilaterally.

Investigations revealed a normal hemogram, liver and kidney function tests. The metabolic screen including arterial blood gas, lactate, ammonia, acylcarnitine profile and urine gas chromatography for organic acids revealed no abnormalities. The electroencephalogram showed diffuse low voltage slow background activity. The muscle biopsy showed reduced cytochrome c oxidase (COX) staining. Facilities for biochemical testing for respiratory chain enzymes in the muscle biopsy were not available. The MRI of the brain showed acerebral leukoencephalopathy involving most of the cerebral white matter with extensive involvement of the corpus callosum and relative sparing of the subcortical U-fibers (Figure 1). There were also cystic changes in the white matter with posterior predominance. The brainstem and cerebellum were spared. The diffusion weighted images showed partial diffusion restriction especially in the rim of the lesions. The MRI findings were suggestive of *APOPT1* cavitating leukoencephalopathy.

APOPT1 exomic sequences were amplified by PCR from total DNA samples obtained from the proband, his mother and his father, using oligonucleotides annealing close to the intron-exon boundaries, as described (1). No PCR product was obtained when trying to amplify exon 3 with the patient DNA (Figure 2A). Next, in the attempt to map the possible *APOPT1* exon 3 deletion in the patient's DNA, a PCR product was amplified using oligonucleotides annealing in regions of intron 2-3 (starting at position 103,572,229 in chromosome 14; Forward) and intron 3-4 (starting at position 103,587,205 of chromosome 14; Reverse), respectively (Figure 2D). A product of approximately 2.2 kb was amplified using the patient and his parents genomic DNAs (Figure 2B), while this product was absent in two control DNA samples (Figure 2B). Another subject, designated as S4 in (1), had previously been reported to carry a homozygous deletion of

APOPT1 exon 3, predicted to generate a truncated protein (p.Glu121Valfs*4). In order to determine if we were dealing with the same pathogenic variant as in the Indian patient described in this report, we analyzed DNA from S4 in the same way. A PCR fragment of a slightly different size (≈ 2.6 kb) was amplified (Figure 2C). In wild-type samples, the hypothetical PCR product should have had a size of 15 kb. The analysis of the sequence showed that there is a 26-bp identical direct repeat region in intron 2-3 and in intron 3-4, where a homologous recombination event occurred, resulting in a 12,820 bp deletion within the *APOPT1* gene (Figure 2D). This deletion maps from position 103,573,702 to position 103,586,522 (NC_000014.9) in chromosome 14, eliminating the *APOPT1* exon 3 (Figure 2D). The patient was homozygous for the deleted allele (Figure 2), while both parents were heterozygous for the wild-type and the deleted allele. S4 carried a deletion in the same genetic area, although in this case the deletion spanned 12,407 bases (from positions 103,573,382 to 103,585,789), which also includes exon 3 together with most of intron 3-4 (Figure 2D). Similarly, there was a 6-bp identical direct repeat flanking the deleted sequence.

The child was started on a mitochondrial cocktail regimen of thiamine (300 mg/day), riboflavin (100 mg/day), Co-enzyme Q (5 mg/kg/day) and carnitine (100 mg/kg/day). He was also advised supportive care in the form of levetiracetam for seizures, baclofen to reduce the spasticity and physiotherapy. Two weeks after starting the treatment, he showed some improvement in the form of recognizing his parents. However the motor abnormalities did not improve. Genetic counseling was done. The parents were explained about the autosomal recessive inheritance pattern and its implications. The parents were also offered molecular genetic testing of the siblings to find out the carrier/ disease status however they refused the testing. The patient died at home three months later from aspiration pneumonia.

Discussion

Our patient presented with subacute onset neuroregression after an attack of measles. Initially diagnostic hypotheses included measles encephalitis and acute disseminated encephalomyelitis, which are known complications of measles.(2, 3) However, the MRI showed symmetrical white matter abnormalities and a peculiar pattern of cavitating leukodystrophy with posterior predominance, which pointed towards the diagnosis.

Recently, Melchionda et al. demonstrated *APOPT1* pathogenic variants to be responsible for mitochondrial disease associated with profound COX deficiency and distinctive neuroimaging features. This gene was identified as part of a project aimed at identifying novel genes responsible for COX deficiency. *APOPT1* pathogenic variants were found in three individuals from independent cohorts of subjects with isolated COX deficiency and subsequently in three additional unrelated children on the basis of a distinctive brain MRI pattern.

The clinical features in these 6 patients varied widely from acute presentation with neuroregression in late infancy to subtle neurological signs presenting in adolescence. The acute presentations were characterized by loss of milestones, seizures, and pyramidal signs rapidly evolving into spastic tetraparesis.(1) Our patient had a similar presentation after an attack of measles. Out of the six previously reported cases, 2 had an onset after a febrile illness. (1) Disease manifestation as a result of stress after a febrile illnesses or infections is a well known feature in inherited metabolic diseases.

In all of the previously reported cases, the clinical course subsequently tended to stabilize and in several patients marked recovery was observed over time. Our patient however had a severe illness, which resulted in his death around 6 months after the onset of symptoms. Treatment

remains supportive; CoQ10 and vitamin supplementation may have a role but this has not been well studied.

A characteristic neuroimaging pattern has been noted with *APOPT1* consisting of early and rapid onset of cavitating white matter abnormalities predominantly in the posterior areas of the cerebral hemispheres and corpus callosum.(1) In severe cases such as in our patient, the white matter abnormalities extend into the frontal and temporal lobes as well as the anterior part of the corpus callosum. Interestingly, it was the recognition of a distinctive MRI pattern in our case that prompted us to targeted genetic testing. Specific MRI white matter abnormality patterns have also been reported in other mitochondrial leukoencephalopathies such as leukodystrophy associated with *DARS2* and *AARS2* mutations.(4, 5) The muscle biopsy shows reduced histochemical cytochrome c oxidase staining and reduced complex IV activity on biochemical respiratory enzyme testing.(1)

The *APOPT1* gene encodes a protein that is targeted and localized within mitochondria. It was initially identified as being overexpressed in atherosclerotic smooth muscle in apolipoprotein E-deficient mice (6). This initial study and a subsequent one assigned a pro-apoptotic role to the protein because its overexpression induced PTP-dependent apoptotic cell death, which could be prevented by activating the Akt pathway.(6, 7) However, no induction of apoptosis or effects on cell viability or growth in different tumor and primary cell types were observed in independent *APOPT1* overexpression studies (1 and M. Zeviani unpublished data). In contrast, acute *APOPT1* knock-down in fibroblasts was associated to a decrease in cell viability (1). The biochemical hallmark in subjects carrying severe mutations in *APOPT1* is isolated COX deficiency, pointing out to *APOPT1* as a factor necessary for COX biogenesis. Its exact molecular role is still under investigation.

In conclusion, we describe a child who presented with subacute neuroregression and neuroimaging pattern of posterior dominant cavitating leukodystrophy who had mutations in the *APOPT1* gene. The recognition of this MRI pattern is helpful for targeted genetic testing.

Ethical considerations: Written informed consent was taken from the parents for writing up and publication of this case. Genetic testing for the patient was done as part of the APOPT1 research project funded by MRC (MRC-QQR grant 2015-2020 and ERC advanced grant ERC FP7-322424). The project has IRB approval.

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Figure Legends

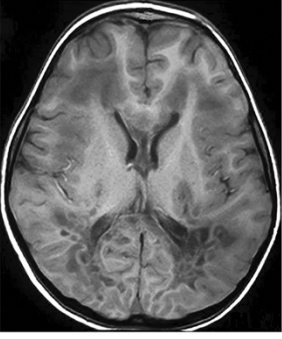
Fig 1A and B

Axial T1- (Fig 1A) and T2-weighted (Fig 1B) images at the level of the basal ganglia showing diffuse white matter signal abnormality with sparing of the subcortical U-fibers. The abnormal white matter has a low signal on the T1-weighted image (A) and a high signal on the T2-weighted image (B). There is involvement of the corpus callosum and posterior limb of the internal capsule with sparing of the anterior limb. The T1-weighted image shows there are cystic changes in the white matter with posterior predominance.

Figure 2. (A) PCR amplification of the exonic region for *APOPT1* exon 3, which was impossible to amplify in the patient sample (P) in contrast to the mother (M) or father (F) DNA samples. Bl: Negative control (Blank); Mk: Molecular weight marker (1 kb Plus Molecular Ladder, Invitrogen). (B) PCR amplification with the forward primer mapping in intron 2-3 and the reverse primer mapping in intron 3-4, respectively. The amplification produced a 2,156-bp band in the subjects of study, while it was absent in two non-deleted DNA control samples. Taken together, the results indicated homozygosity for the deletion in the patient and heterozygosity in both parents. Bl: Negative control (Blank). (C) PCR amplification with the same primers as in B, comparing the 2,569-bp PCR product in subject S4 (1) and the 2.2 kb band in the subject of this study (P). Again, the band was absent in control DNA (C1). (D) The *APOPT1* gene is composed of five coding exons. The positions of the forward (Fw) and reverse (Rv) primers used to generate the PCR products in panels B and C are indicated. The extent of the deletion found in patients P (this report) and S4 (1) is also indicated. The exon composition and coding regions of the wild-type and mutated *APOPT1* mRNAs are represented

schematically. Deletion of exon 3 predicts a frameshift and a truncated protein of 125 amino acids (p.Glu212Valfs*4) vs. the 206 amino acids of the wild-type protein, according to the reference cDNA sequence NM_032374.

A



B

