11th International Symposium

Sociedade Portuguesa de Doenças Metabólicas

ADULT-ONSET FORM IN VLCAD DEFICIENCY: SEVEN CASES

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INTRODUCTION

Very long chain acyl-CoA dehydrogenase deficiency (VLCADD, MIM 201475) is an autosomal recessive disorder characterized by impaired mitochondrial β-oxidation of fatty acids with a chain length between 14 and 18 carbons. The prevalence of VLCAD deficiency in Portugal is 1/101,613. VLCADD has three forms of clinical presentation: severe early-onset; intermediate with childhood onset and adult-onset, of mild severity, characterized by exercise intolerance, myalgia and recurrent episodes of rhabdomyolysis (1). The VLCAD gene (*ACADVL*) contains 20 exons that encode a 655-amino-acid protein and it is located on chromosome 17p13. More than 116 mutations have been identified in the literature (HGMD).
The development of electrospray ionization tandem mass spectrometry (MS/MS) has allowed beyond the screening of neonatal forms a marked improvement on diagnosis of the adult onset form, through the analysis of acylcarnitine profiles from blood spots, using C14:1 as primary marker. The molecular study of *ACADVL* gene allowed the confirmation of the patients.

Table 1- Clinical classification of the seven VLCADD patients. Family A-patients 1 and 2 are brothers; Family C-patients 4 and 5 are brothers.

	Family A		Family B	Family C		Family D	Family E
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age/sex	13 Years Female	19 Years Male	51 Years Male	11 Years Male	20 Years Male	14 Years Female	63 Years Female
Clinical phenotype	-Severe generalized muscle pain -Dark urine after prolonged physical effort -CPK↑ -mioglobin↑ -mioglubinuria	-Episodes of muscle pain -Dark urine after physical effort of moderate intensity since the age 17 -At age 18 hospitalization due to not elucidated rhabdomyolysis	- Experienced the first of several recurrent episodes of myalgia at 20 years -Muscle weakness -Rhabdomyolysis following prolonged exercice -CPK↑	-Exercise intolerance -Dark urine	-Myalgia -Rhabdomyolysis	- Rhabdomyolysis	-Rhabdomyolysis -Myalgia

PATIENTS AND METHODS

The authors report seven individuals from five families with clinical symptoms and ages between 11 y-63y (Table 1). Blood spot samples are collected in Whatman 903 filter paper.
Acylcarnitines were analysed as butyl esters on an API 2000 triple quadrupole tandem mass spectrometer (AppliedBiosystems, Sciex) with an ion spray device, as previously described with minor modifications (2).
Genomic DNA was extracted from blood of patients and their parents by standard methods. All 20 exons of the *ACADVL* gene (3), and respective flanking regions, were amplified by PCR, using newly designed primers and was performed on an automatic sequencer (ABI Prism 3130XL).

RESULTS

The analysis by tandem mass spectrometry of the acylcarnitines profile in seven individuals with clinical symptoms revealed accumulation of tetradecenoyl carnitine (C14:1), suggesting the diagnosis of VLCADD. The molecular characterization allowed the identification of mutations in all cases (Table 2), thus confirming this diagnosis.

Table 2- Biochemical and molecular data from the seven VLCADD patients.

	C14:1 µM	C14:2 µM	C14:1/C16	C14:1/C12:1	Genotype	Deduced effect
Ref. value	<0.18	<0.08	<0.19	<2.60		
Patient 1	0.35	0.23	0.30	4.38	c.[1500 _1502delCCT]+ [1500 _1502delCCT]	p.[L500del]+[L500del]
Patient 2	0.25	0.01	0.10	1.25	c.[1500 _1502delCCT]+ [1500 _1502delCCT]	p.[L500del]+[L500del]
Patient 3	7.17	0.67	2.43	15.42	c.[1097G>A]+[1097G>A]	p.[R366H]+[R366H]
Patient 4	0.47	0.17	0.46	5.88	c.[1500 _1502delCCT]+ [1500 _1502delCCT]	p.[L500del]+[L500del]
Patient 5	0.22	0.10	0.16	1.70	c.[1500 _1502delCCT]+ [1500 _1502delCCT]	p.[L500del]+[L500del]
Patient 6	1.27	0.30	1.72	9.77	c.[187_192insA]+[1097G>A]	p.[P65Tfs*7]+[R366H]
Patient 7	0.25	0.09	0.52	3.64	c.[1500 _1502delCCT]+ [1500 _1502delCCT]	p.[L500del]+[L500del]

DISCUSSION / CONCLUSION

In spite of the heterogeneity of genotype usually associated with VLCADD, in these cases we found the same mutation (p.L500del) in 10/14 allels and another mutation p.R366H in 3/4 reaming allels. This fact may indicate that this mutations are commonly associated with mild VLCADD form in the Portuguese population.
When rhabdomyolysis is present in a patient, and after differential diagnosis exclusion, it is important to consider the possibility of a VLCAD deficiency.
However late-onset forms may be undetectable by acylcarnitine profile in asymptomatic period, and whenever possible samples should be taken in in crisis period. If VLCADD is considered suspicious the molecular analysis of *ACADVL* should be performed even in the presence of a normal acilcarnitines profile , to avoid a late diagnosis.

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