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Twenty Years of Newborn Screening for MCADD in Portugal: genetic data

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INTRODUCTION

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessive inherited metabolic disorder that affects fatty acid oxidation metabolism. Before the introduction of newborn screening (NBS), approximately 20–25% of infants with MCADD died suddenly during the first episode of metabolic decompensation. However, since the implementation of NBS using tandem mass spectrometry (MS/MS) in the 1990s, this mortality rate has significantly decreased due to early diagnosis and appropriate management.

Most cases present the most common c.985G>A mutation in ACADM gene, while a few patients carry other rare mutations. In Portugal, MCADD has been included in the newborn screening program since 2004 and is the most frequently diagnosed inborn error of metabolism detected through this program (1), with an incidence of 1 in 6,433.

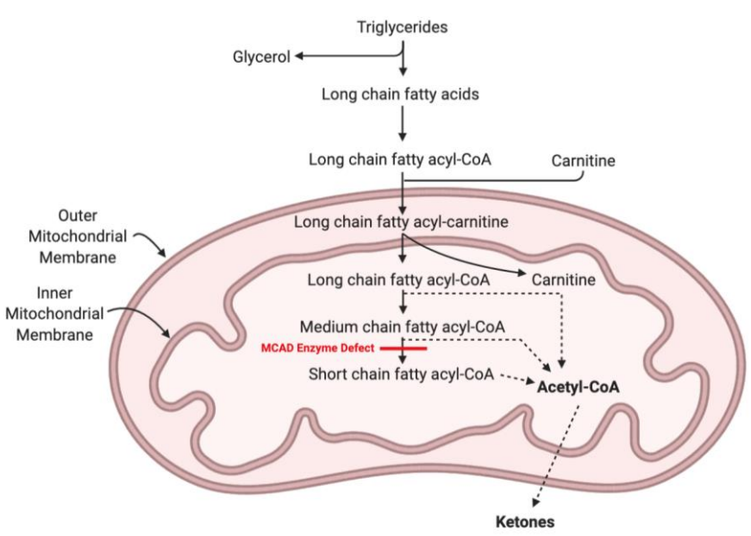

Figure 1: Fatty acid β-oxidation metabolic pathway indicating the MCADD block.

MCADD impairs the ability to break down medium-chain fatty acids for ketone and ATP synthesis (figure 1). Is one of the key enzymes involved in mitochondrial fatty acid β-oxidation, which drives hepatic ketogenesis—a major energy source when hepatic glycogen stores are depleted during prolonged fasting or periods of increased energy demand. Once diagnosed, the prognosis is favorable with the implementation of frequent feedings to prevent extended fasting. Early identification and treatment of patients with MCADD are expected to prevent long-term complications. The metabolic phenotype of MCADD is characterized by the elevated excretion of diagnostic compounds C6-C8 dicarboxylic acids, suberylglycine and hexanoylglycine acid in the urinary organic acids profile and the presence of abnormally elevated blood levels octanoylcarnitine (C8) and ratio C8/C10 determined by tandem mass spectrometry (MS/MS).

PATIENT AND METHODS

Approximately 1,762,713 newborns were screened for MCAD deficiency between October 2004 and January 2025, using MS/MS to detect elevated octanoylcarnitine (C8) levels and an increased C8/C10 ratio.

The cutoff value for a positive MCAD was established by the Portuguese Newborn Screening laboratory (2).

Blood spot samples from newborns are collected between day 3 and 6 in Watman 903 filter paper. Acylcarnitines in samples are analysed as butyl esters (3) by using a triple quadrupole tandem mass spectrometer API 4000 (AB, Sciex) and quantified using internal standards from Cambridge Isotope Laboratories.

Gene *ACADM* that encodes the enzyme MCAD were studied by reported methods.

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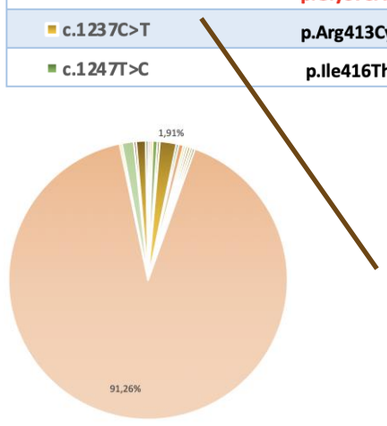
RESULTS

Over the 20 years period, a total of 274 newborns with high values of C8 and C8/C10 ratios were identified and reported to a clinical reference center for metabolic diseases. Out of these, biochemical and molecular follow-up confirmed the diagnosis of MCADD in 273 cases. In one case, no MCAD variants were detected, and in 90 cases, molecular characterization was not available. Among the remaining 183 cases studied at our unit, 160 (87%) were homozygous for the c.985G>A mutation, while 23 were identified as compound heterozygotes (figure 2). Of these, 14 cases carried the c.985G>A mutation along with another mutation, while 9 cases presented with two distinct mutations. Additionally, seven novel variants were identified in this cohort: c.94G>C, c.113G>C, c.214G>T, c.532A>T, c.974A>G, c.1133G>A, and c.708+1G>A. The novel nonsense and frameshift mutations are considered pathogenic variants, while the novel missense mutations have predicted effects as show in figure 2. The variants c.94G>C and c.113G>C were identified in the same case in heterozygosity with the most common mutation, c.985G>A. It was not possible to perform segregation analysis for this case.

Variants	Protein	Types	Class	Clinvar	# allelo (N=366)
c.94G>C	p.Glu32Gln	missense	B	unreported	1
c.113G>C	p.Ser38Thr	missense	VUS	unreported	1
c.199T>C	p.Tyr67His	missense	PV	Variation ID: 3597	2
c.214G>T	p.Glu72*	nonesense	PV	unreported	1
c.250C>T	p.Leu84Phe	missense	PV	Variation ID: 226097	7
c.532A>T	p.Lys178*	nonesense	PV	unreported	1
c.617G>A	p.Arg206His	missense	PV	Variation ID: 92268	2
c.653C>G	p.Ala218Gly	missense	PV	Variation ID: 1404546	1
c.708+1G>A	p.?	splicing	PV	unreported	1
c.778_782delGAAAA	p.Glu260Cysfs*5	frameshift	PV	Variation ID: 1457250	1
c.890A>G	p.Asp297Gly	missense	LPV	Variation ID: 2679879	1
c.974A>G	p.Glu325Gly	missense	PV	unreported	1
c.985A>G	p.Lys329Glu	missense	PV	Variation ID: 3586	334
c.1045C>T	p.Arg349*	missense	PV	Variation ID: 189016	1
c.1189dupT	p.Tyr397Leufs*5	frameshift	PV	Variation ID: 226109	5
c.1133G>A	p.Gly378Asp	missense	LPV	unreported	1
c.1237C>T	p.Arg413Cys	missense	PV	Variation ID: 850051	4
c.1247T>C	p.Ile416Thr	missense	LPV	Variation ID: 203543	1



Variants	Mutation Taster	PolyPhen2	SIFT
c.94G>C	Benign	Benign	Tolerated
c.113G>C	Benign	Possibly D	Tolerated
c.974A>G	Deleterious	Probably D	Deleterious
c.1133G>A	Deleterious	Probably D	Tolerated

Figure 2: Variants spectrum in ACADM gene identified in our patients. Novel variants are given in red.

CONCLUSION

Newborn screening has been crucial for identifying and managing MCADD in Portugal. Our study confirms the c.985G>A mutation as the most frequent pathogenic variant, consistent with previous reports. The identification of seven novel mutations expands the spectrum of known variants, underscoring the importance of comprehensive genetic analysis. These findings reinforce the importance of newborn screening in early diagnosis and intervention, while also contributing to a deeper understanding of the genetic diversity of MCADD, with implications for genetic counseling and long-term management.

REFERENCES