

A new form of CPT2 deficiency in a 15-year-old patient

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ABSTRACT

In this article we present the case of a 15-year-old patient diagnosed in Romania with a new form of CPT2 deficiency (carnitine-palmitoyl-transferase). This diagnosis was established by genetic and functional tests. In this way we want to show a possible etiology of serious cases of rhabdomyolysis and to indicate a treatment for these cases. The diagnosis was made late because the clinical picture was not a specific one, the manifestations being long ignored or misinterpreted as behavioral disorders. We also want to draw attention to a new gene configuration that can sometimes lead to extremely severe crises involving life-threatening.

Keywords: rhabdomyolysis, hyperammonia, heart disease, acute renal failure, hypoglycemia

INTRODUCTION

Lipid metabolism falls into the category of intermediate metabolism which is responsible for producing the necessary energy of the body's performance. It consists of several parts. The first part comprises the system of decomposition of ingested fats up to the level of long chain fatty acids, provided by digestive enzymes.

Subsequently these acids are taken over by the transport system of long-chain fatty acids through the mitochondrial membrane made by three enzymes (CPT1, CPT2, CACT).

The last part is represented by mitochondrial beta-oxidation which is served by six enzymes (VLCAD, LCHAD, trifunctional enzyme, MCAD, SCAD, SCHAD). They are intended to remove from the fat acid chain a molecule of acetylsalicylic-CoA (1).

A dysfunction of these processes will have three effects. The first effect is a build-up of fatty acids that can no longer be degraded, depending on the enzyme involved, long, medium or short chain fatty acids may accumulate (2). Beta-oxidation activates pyruvate-carboxylase and thus activates neoglucogenesis (3). A decrease in beta-oxidation function will affect the functioning of neoglucogenesis and thus generate hypoglycemia.

A lack of ketone bodies that will cause the heart muscle and brain to lack an alternative energy substrate in the event of hypoglycemia (4). However, this is also useful for diagnosis, as we may suspect a disorder of fatty acid metabolism when we have a patient with non- or hypoketotic hypoglycemia.

There is also a peroxysome beta-oxidation that degrades very long chain fatty acids. This should not be confused with the mitochondrial one, the clinical picture being completely different (5).

Of all these entities, the most dangerous are those that lead to the accumulation of long-chain fatty acids. They are toxic to both skeletal and cardiac muscles. Their accumulation leads to severe rhabdomyolysis at risk of acute renal failure and heart attack or by direct myocardial toxicity or secondary hyperpotasemia rhabdomyolysis. These entities include CPT1 deficiency, CPT2 deficiency and CACT which together form the system of transport of carnitine through the mitochondrial membrane; primary carnitine deficiency, and from beta-oxidation disorders we list the deficiency of LCHAD, VLCAD, trifunctional enzyme and MADD. The CPT2 deficiency comprises three forms, myopathic, neonatal and infantile. Just over 300 cases are reported worldwide, of which 86% are cases of CPT2 deficiency muscle form, 8% are infantile cases and only 6% are neonatal cases.

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The clinical picture is dominated by rhabdomyolysis of varying severity. It is accompanied by long post and non-cetotic post hypoglycemia. Commonly associated with these signs and hyperamoniaemia, generally mild to moderate but which can sometimes be severe.

A redoubtable complication is heart damage that can be of the type of heart failure or the appearance of rhythm disorders with fatal risk. Also, secondary to rhabdomyolysis, an acute renal failure may be established which may require the initiation of dialysis.

CASE PRESENTATION

Methodology

The diagnosis was suspected following a severe seizure of rhabdomyolysis. NgS (next generation sequencing) technique was used to sequence genes involved in metabolic rhabdomyolysis. Since the result was not a safe one, functional and enzymatic tests were necessary to establish with certainty the etiology.

History

The case is that of a male teenager who had no particular problems until the age of 7. From the age of 7 the mother notes that the boy has a lower tolerance to effort compared to other children and an increased food appetite.

At the age of 10, the first episode of rhabdomyolysis is noted, but it is not further explored. Hypoglycemia outbreaks not identified.

At the age of 13 he has a new episode but much more severe for which he requires hospitalization. At the time of hospitalization, the patient experienced precordial pain, fever, breathing disorders and muscle pain. He's immediately hospitalized.

Routine investigations have revealed a severe rhabdomyolysis (CK > 80.000 U/l) heart damage (CKMB = 1,240 mg/dl, hepatic cytolysis, myoglobulinuria and hyperuricemia). The crisis is remitted under symptomatic treatment and hydration, without affecting renal function. Upon discharge, however, the patient retains a slight rhabdomyolysis and a discrete increase in myocardial enzymes but these were not clinically manifested at that time.

The evolution of the relevant parameters during hospitalization is shown in Table 1.

Rabdomyolysis is preserved, but accompanied by more or less intense muscle pain, which is why the suspicion of metabolic myopathy is raised, infectious and autoimmune causes being excluded.

Over a year, the patient makes a new crisis of rhabdomyolysis accompanied by muscle pain but less se-

vere. Following this new crisis, a consultation of congenital diseases of metabolism is requested.

At the time of the consultation, the parameters were those shown in Table 2.

TABLE 1. Paraclinical data during hospitalization

Date	CPK	AST	ALT	K
09/04/2018	81,154	1,990	528	5.3
10/04/2018	72,060	1,882	5,471	3.6
11/04/2018	38,070	1,471	437	4.2
12/04/2018	12,099	665	252	3.2
13/04/2018	7,437	609	327	4.7
16/04/2018	832	83	117	6.1
19/04/2018	1,548	93	132	4.6

TABLE 2. Paraclinical values at the time of the specialist consultation

Value	Parameter
535	CPK
33	CKMB
37.2	AST
40.3	ALT
91.6	NH3

An emergency therapy to normalize the level of ammonia with sodium benzoate has been initiated, leading to a normalization of its level. Muscle enzymes CPK and CKMB also decreased following a lifestyle based mainly on avoiding physical exertion. A prophylactic therapy with Carnil has also been initiated.

The association between severe rhabdomyolysis and accentuated by febrile outbreaks, hyperamoniaemia, liver and cardiac damage raises the suspicion of a congenital disease of metabolism.

Differential diagnosis

The main entities that give this clinical picture are:

- Disorder in beta-oxidation of long-chain fatty acids
- Disorder in carnitine metabolism
- CPT1, CPT2, CACT deficiency
- Glycogenosis II, III, IV, V, VII, IX
- Mitochondrial cytopathies

Establishing the certain diagnosis

A gene sequencing by NGS technique of genes involved in metabolic rhabdomyolysis was performed, the result being shown in Figure 1.

A first mutation was discovered, in heterozygous form, in the CPT2 gene (c.1862T>C). This mutation was not known until now, it does not exist in population databases and has not been cited in people with CPT2 deficiency.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
CPT2	c.1862T>C (p.Ile621Thr)	heterozygous	Uncertain Significance
CPT2	c.604T>A (p.Tyr202Asn)	heterozygous	Uncertain Significance
POLG	c.1402A>G (p.Asn468Asp)	heterozygous	Uncertain Significance
SLC22A5	c.1255C>G (p.Leu419Val)	heterozygous	Uncertain Significance
GAA	c.1726G>A (p.Gly576Ser)	heterozygous	Benign (Pseudodeficiency allele)
GAA	c.2065G>A (p.Glu689Lys)	heterozygous	Benign (Pseudodeficiency allele)

FIGURE 1. The result of gene sequencing

A second mutation in the CPT2 gene was identified, namely c.604T>A, also in heterozygous form. However, there are no studies indicating the effect of missense on the structure and functionality of the protein and, as in the first case, this mutation is unknown, it does not exist in population databases, nor has it been cited in patients with CPT2 deficiency.

As known, the CPT2 deficiency is manifested by severe rhabdomyolysis, hyperammonia and heart damage, which may explain the clinical picture, but since the pathogenic effect of the two mutations has not been proven, the diagnosis cannot be validated solely on the basis of this result.

Interestingly, a third mutation, also in heterozygous form, was discovered in the SLC22A5 gene (c.1255C>G). This gene is associated with the primary deficit of carnitine. The mutation is a known one, it exists in population databases, but has not been cited in patients with primary carnitine deficiency.

The clinical picture of the primary carnitine deficiency is the same as that of the CPT2 deficiency and matches the clinical-biological picture of the patient.

POLG deficiency is a known cause of mitochondrial cytopathy but, since the patient's clinical picture does not overlap with those found in POLG deficiency, we considered the discovered mutation to have no significance.

Since the genetic result is not conclusive, functional and enzymatic tests were required.

A carnitine profile was developed, which highlighted an increased level of urinary carnitine, with a higher value than serum (table 3).

TABLE 3. Carnitines' profile

Parameter	Value	Reference range
Urinary free carnitine	49.8 mg/24h	15.2-41.2
Total serum carnitine	8.52 mg/l	4.67-9.37
Serum free carnitine	6.76 mg/l	3.96-8.21
Acilcarnitine ratio	0.26	< 0.40

However, we can note that serum levels represent > 80% of urinary level, which makes a primary deficit of carnitine unlikely since in this disease the serum level of carnitine is very low, < 50% of the urinary level. So, we considered that it should be taken into account that the patient has a new form of CPT2 deficiency, with a gene mutation never seen before. In order to determine whether it is a *cis* or a *trans* form, we decided to sequence the CPT2 gene through the NGS technique of both parents. The results are presented in figure 2A,B.

It is noted that each parent is positive for an identified mutation of the patient, and the father also has the POLG gene mutation identified in the child, indicating that the mutations of the CPT2 gene are in *trans* form, and the patient is only the carrier of the POLG mutation, which has no pathogenic effect.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
CPT2	c.1862T>C (p.Ile621Thr)	heterozygous	Uncertain Significance

FIGURE 2A. Mother

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
CPT2	c.604T>A (p.Tyr202Asn)	heterozygous	Uncertain Significance
POLG	c.1402A>G (p.Asn468Asp)	heterozygous	Uncertain Significance

FIGURE 2B. Father

FIGURE 2A, B. Test results demonstrating allelic segregation

Test results			
Carnitine palmitoyltransferase 2 (CPT2)		in LYC, sample: 20E0081	
	nmol/(min.mg protein)	Reference values	
Activity	1.7	9.0	22.6
Reduced carnitine palmitoyl-CoA transferase 2 (CPT2) activity			

Conclusion	
The activity of carnitine palmitoyl-CoA transferase 2 (CPT2) in lymphocytes of the patient was reduced compared to the reference values. This result confirms that the patient has a deficiency of CPT2.	

FIGURE 3. Residual enzyme activity of carnitine-palmitoil-transferase

Since the *trans* form is pathogenic, the probability of these two mutations causing a real enzyme deficiency is increased. This effect was verified by measuring the residual enzyme activity of the enzyme CPT2, the result being shown in Figure 3.

A clear enzyme deficiency is thus observed, which validates the diagnosis and certifies the pathogenic effect of these two new mutations.

The criteria on the basis of which the diagnosis was suspected were therefore the association between severe rhabdomyolysis, hyperamoniaemia and noncetotic hypoglycemia.

Treatment and evolution

The first therapeutic gesture initiated was the start of the therapy with an ammonia purifier. This was sodium benzoate at a dose of 3 g four times a day. The choice was motivated in particular by pecuniary, sodium phenylbutyrate being difficult to reach. The value of ammonia normalized very quickly, without the need to associate the protein restriction.

Carnil treatment has been stopped, as it has no usefulness in CPT2 deficiency.

The nutritional regime is very important in this disease and it consists in reducing the intake of long-chain fats so that they do not exceed 30% of the caloric intake. At the same time, given that the patient is more than 120 kg at 190 cm, a reduction in calorie intake has been attempted. Therapeutic compliance was not very good, however, but even so, the values of muscle and liver enzymes decreased considerably (table 4).

TABLE 4. Dynamics of laboratory test values under treatment

Data	CK	CKMB
08/07/2019	485 (VN<308)	44 (<24)
13/12/2019	1.399 (VN<308)	59 (<24)
25/03/2020	351 (VN<308)	-

An important focus was also placed on the prevention of infectious diseases through influenza vaccination and the avoidance of potentially infectious contacts.

Hypoglycemia prophylaxis was carried out with the help of Maizena maize starch administered after each meal. Hypoglycemia has not been recorded since initiation of this treatment.

An emergency protocol was given to the patient in the event of a new seizure.

Therefore, the most important element of the treatment of this patient is the diet that follows the limitation of lipid intake.

DISCUSSION

Fatty acid metabolism disorders can be grouped into several categories:

- intramitochondrial beta-oxidation disorders
- primary carnitine deficiency (OCTN2)
- disturbances in carnitine transport paths (CPT1, CPT2 and CACT)

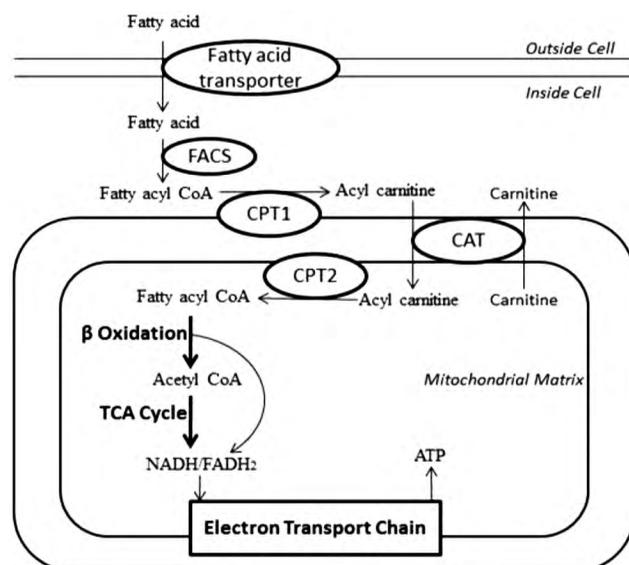


FIGURE 4. Scheme of beta-oxidation of fatty acids and accessory ways (6)

Beta-oxidation disorders are the main cause of metabolic rhabdomyolysis. Since the prognosis of these diseases is a good one if the diagnosis is established quickly and the treatment is instituted quickly, the rapid recognition of these cases is extremely important. However, abnormalities in the ancillary pathways of beta-oxidation such as the transport of carnitine through the mitochondrial membrane or through the epithelium of the kidney are an important entity. The clinical picture of these diseases is identical to that of beta-oxidation disorders, differentiation only on clinical criteria being practically impossible.

Both in beta-oxidation disorders and in those of related routes, long-chain fatty acids are accumulated which are toxic to the skeletal muscle and to the myocardium. These acids are no longer degraded in the process of intramitochondrial beta-oxidation or because they can no longer cross the mitochondrial membrane as happens in related horse disorders or penetrate into the mitochondria but the enzymes involved in their degradation no longer work, as happens in beta-oxidation disorders. The results of these disorders are a decrease in the energy production required for the functioning of neoglucogenesis, which leads to hypoglycemia and a decrease in the production of ketone bodies that would normally have a protective role for the brain and heart during hypoglycemia. In addition, the toxic effect on the striated muscles produces an important rhabdomyolysis that can lead to acute renal failure or even cardiac arrest. Also, in this pathology abnormalities of heart rhythm that can be fatal are observed.

Another complication is inhibition of the urea cycle, which will produce a hyperamoniathat may be dangerous (7).

The purification of these acids is done with the help of carnitine, but in the disorders of carnitine transport this treatment is not useful, the carnitine molecule cannot reach the level of mitochondria where these acids accumulate. Particular caution is required at the time of initiation of treatment since, in a first phase, by the administration of carnitine, the serum level of long-chain acilcarnitine increases and this increased concentration may induce a pharmacoresistant heart failure. While there is no consensus on how to deal with this problem correctly, it is recommended to introduce carnitine prudently and progressively to a daily acilcarnitine profile to assess the rate of increase in their serum levels. If there is no such possibility, cardiac ultrasound can be used with the study of the ejection fraction.

Another important link of treatment is the blocking of catabolism. As a result of catabolism, long-chain fatty acids are produced, and the rate of produc-

tion exceeds the purification capacity of carnitine. To achieve this, it is necessary to use a high glucose flow (10 mg/kg/min) but it is often necessary to add a continuous infusion of insulin, thus speculating the anabolic effect of this hormone.

Ammonia purifiers (sodium benzoate, sodium phenylbutyrate) are also required to normalize ammonia.

Background treatment focuses on preventing hypoglycemia and avoiding the accumulation of long-chain fatty acids. Particular attention is paid to the diet which should not contain complex fats, their intake should represent a maximum of 30% of the caloric intake. This, combined with the administration of carnitine as an acilcarnitine purifier, can achieve good metabolic control and prevent seizures.

Hypoglycemia can be prevented with Maizena corn starch.

Another way to achieve this regime is to use special products. Thus, according to a French team, special Monogen formulas containing 90% medium chain fatty acids (AGLM) can be used as the child grows and we can no longer use this formula, other sources of AGLM such as Liquigen can be used. In this milk can be added walnut oil that will ensure the intake of essential fatty acids. The remaining calories will be provided with carbohydrates (8).

CPT2 deficiency is a very rare disorder, with about 350 cases recorded in the world so far. Three forms are recorded: neonatal, hepatomusculocardiac and myopated. This small number of patients makes it very difficult to establish a therapeutic protocol.

Most experts agree that diet is the best solution alongside proper emergency management. Prolonged fasting should also be avoided. Drug therapy has not been shown to be effective (9). Other sources suggest the use of high-dose carnitine (100 mg/kg) po during acute release but the effectiveness is questionable (10).

Particular attention is paid to hydration during the crisis. This hydration is intended to prevent acute renal failure secondary to rhabdomyolysis. It is associated with a glucose infusion to block cleavage of fat (10).

According to a French community, however, essential in managing the crisis is the blocking of the cleavage of fat, with hydration becoming a priority only if CK > 20,000 U/l (11).

According to a study by a community, the main organ affected during these acute phases is the kidney. Prompt therapy as described above, however, led to the recovery of most patients and prevented the development of multiple organ failure (12).

Another important element is the avoidance of certain medicines during the crisis, they can aggravate rhabdomyolysis. These are:

- depakin
- ibuprofen
- diazepam in high doses
- anesthetics used for general anesthesia (13).

In terms of the type of fat used in background therapy, it is represented by medium chain fats that must provide 30% of the necessary caloric intake. They have the advantage that they do not require the presence of carnitine to penetrate the mitochondria and will not accumulate in this way (8).

An interesting case was communicated in an article by a community of doctors from Croatia (14). They presented the case of an adult woman suffering from this disease. The medical team used as a therapeutic method only a regimen based on frequent meals, without the use of an ammonia or Maizena purifier. Under this treatment the patient has fully recovered and remains asymptomatic.

Increased attention deserves genetic analysis of the family members of these patients. A study by a group of doctors in Austria describes the case of a family in which no fewer than 24 affected members have been identified (15). In the case of two of these members the diagnosis was postmortem.

Physicians experiencing patients with rhabdomyolysis should also know that people affected by a CPT2 deficiency may be completely asymptomatic and have normal laboratory learnings between rhabdomyolysis for several years, making the diagnosis extremely difficult. This is the case of a South American family who, although they had rhabdomyolysis, was diagnosed late as they could not be evaluated dur-

ing them (16).

A private discussion deserves the neonatal form. This is frequently fatal even in the presence of appropriate treatment. It is essential to quickly recognize it and initiate catabolism-blocking therapy using high-flow glucose infusion (> 10 mg/kg/min) as well as ammonia treatment therapy. Even so the prognosis remains reserved, and death occurs frequently (17).

CONCLUSIONS

The main peculiarity of this case is that it is the first time that a CPT2 Deficiency is due to this gene binomial. Phenotype-genotype correlation is difficult to achieve and only careful follow-up can provide all the necessary information.

Interestingly, however, the patient survived until the age of 14 without adequate treatment and with few decompensations, with only two being truly serious. Although, at this point the patient's compliance is not very good, he has not had new seizures. All these elements lead us to believe that this gene binomial is not one that indicates a poor prognosis, but a clear conclusion cannot be drawn at this point on this.

The usefulness of publishing this case is not only the fact that two new mutations have been discovered, but in order to raise awareness of pediatricians for this etiology.

Beta-oxidation deficiency and carnitine metabolism abnormalities remain, however, diseases with a good prognosis, so rapid recognition of their manifestations is essential.

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