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# Post-mortem Diagnosis of Pyruvate Carboxylase Deficiency by Exome Sequencing in a Family with three Deceased Children: A Case Report

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**Abstract** **Case Report**

Pyruvate carboxylase (PC) deficiency (OMIM, 266150) is a rare autosomal recessive disorder. It is characterized by developmental delay, failure to thrive, metabolic acidosis, and recurrent seizures. Hypoglycemia is an inconsistent finding. PC deficiency is divided in three clinical subtypes: an infantile form (Type A), a severe neonatal form (Type B), and a benign form (Type C). It is due to biallelic mutations in *PC* gene. In this study, we report the case of a Moroccan nonconsanguineous patient with hypotonia and metabolic acidosis. They had two deceased sibs with same phenotype and no accurate diagnosis. Clinical exome sequencing identified a compound heterozygous mutation c.2278C>T (p.Arg760Trp) and c.2602G>A (p.Gly868Arg) of *PC* gene leading to a post-mortem diagnosis of Pyruvate carboxylase deficiency. The identification of the genetic substrate in the deceased patient confirmed the clinical diagnosis of Pyruvate carboxylase deficiency and allowed appropriate genetic counseling to the family for future pregnancies. This case is the first observationof a post mortem case of familial PC deficiency, and it relates the importance of Next Generation Sequencing in post mortem diagnosis of patients with uncertain diagnosis.

**Keywords:** Post-mortem diagnosis, PC deficiency, *PC* gene, case report.



## INTRODUCTION

Pyruvate Carboxylase (PC) deficiency is a rare autosomal recessive disorder of metabolism. Three distinct clinical presentations of PC deficiency are described. The infantile form (Type A, North American Form) mostly seen in North American Indians, and defined by infantile-onset, lactic acidemia, psychomotor retardation, failure to thrive, hypotonia, nystagmus, convulsions, and death in infancy or early childhood [1-3]. The neonatal form (Type B, or French Form), is characterized by early beginning in life with severe lactic acidosis, hepatomegaly, hypotonia, convulsions, rigidity, and delayed psychomotor development [4,5]. These patients had elevated blood levels of ammonia, citrulline, proline and lysine. Patients usually died in the first months of life. The type C (intermittent/benign form), is characterized by normal or mildly delayed neurologic development and episodes of metabolic acidosis.

The severity of PC deficiency is related to the degree of residual enzyme activity, and also to molecular phenotypes. The diagnosis of PC deficiency is established by identification of PC enzyme deficiency in fibroblasts or lymphoblasts, and identification of biallelic PC mutations. Pyruvate carboxylase is a mitochondrial enzyme that catalyzes the conversion of pyruvate to oxaloacetate. It plays a crucial role in gluconeogenesis, energy production (through Krebs cycle), and in anaplerotic pathways [6].

We present here the first case of post mortem diagnosis of PC deficiency in a Moroccan consanguineous family with three deceased children, and we focus on the importance of Next Generation Sequencing in diagnosis of metabolic diseases.

## CASE REPORT

A young nonconsanguineous Moroccan couple was referred to the Department of Medical Genetics in Rabat because of two deceased children at very young age of 7 days and 2 months (Figure 1). They were the parents of a baby girl, six days old, presenting hypotonia, convulsions, and hyperammonemia. She had generalized hypotonia, hyperammonemia, hyperlactemia, and high levels of pyruvate, alanine, citrulline, and lysine. She had also metabolic acidosis. She died two days after genetic consultation without

prior evaluation. In the family history, she had two brothers with the same phenotype, deceased respectively at three months, and 6 days of life without an accurate diagnosis.

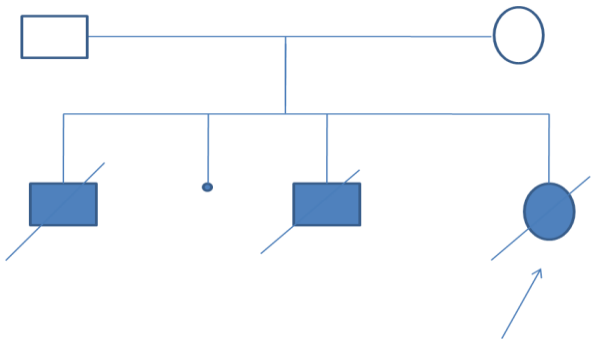

**Fig-1: Pedigree of the family.**

Parents gave written informed consent to the genetic analysis, which was performed in accordance with the Declaration of Helsinki protocols. An ethylene diamine tetra acetic acid (EDTA) blood sample of the proband was performed with parental consent before she died. Because of the significant family history and in order to establish a potential post-mortem diagnosis in the proband, we have chosen to apply the Clinical Exome Solution™ by Sophia Genetics on an Illumina NextSeq-500 platform. Variants with allele frequencies above 2% in GnomAD and ExAC, and variants not predicted to be deleterious were excluded. The established variants were cross-checked with 1000 Genomes Project database, Exome Variant Server, Human Gene Mutation Database, and ClinVar database. A filter was used for virtual panel of genes known to be involved in metabolic acidosis with hyperammoniemia.

A compound heterozygous mutation c.2278C>T (p.Arg760Trp) and c.2602G>A (p.Gly868Arg), in exons 16 and 17 of PC gene were identified. These variants have never been reported in databases and were predicted as pathogens by prediction tools: SIFT, Mutation Taster and Polyphen2. This result confirmed the diagnosis of Pyruvate Carboxylase type B of the proband. The deceased brothers had very probably the same genetic disorder.

## DISCUSSION

PC deficiency is a rare metabolic disorder inherited in an autosomal recessive manner. The birth incidence of PC deficiency is approximately 1:250000 [7]. There are three forms of PC deficiency. The PC deficiency type A (or infantile form) had an increased incidence among the native North American Ojibwa, Micmac, and tribes Cree of the Algonquin-speaking peoples. The type C or intermittent/benign form is characterized by mildly delayed neurologic development and episodic metabolic acidosis. Few patients have been reported [8, 9].

Type B of PC deficiency is the most severe form with neonatal lethal lactic acidosis. This type B has an increased incidence in Europe [9]. The patients had increased lactate-to-pyruvate ratio, and elevated blood concentrations of citrulline, proline, lysine, and ammonia. Blood pyruvate concentrations are usually elevated in PC deficiency type B, resulting in an elevated lactate-to-pyruvate ratio. The majority of patients die in the first three months of life; however, some affected individuals were alive at ages nine and twenty years, probably because of mosaicism [9, 10]. In the family reported here, the three deceased patients had elevated lactate-to-pyruvate ratio, and died before three months of life like majority of reported patients.

The diagnosis of PC deficiency is established by identification of PC enzyme deficiency in fibroblasts or lymphoblasts. In individuals with PC deficiency, fibroblast PC enzyme activity is usually less than 5% of that observed in controls [9]. The diagnosis of PC deficiency can also be established by identification of biallelic pathogenic variants in PC gene. PC gene contains 20 coding exons and four non-coding exons located at the 5'-untranslated region (5'-UTR). Pathogenic variants of coding region and promoter are responsible of 95% of mutations. The p.Ala610Thr mutation was reported in 13 patients originated from of Ojibwa and Cree. The carrier frequency in these populations may reach 1:10 [11].

Ostergaard reported seven patients with PC deficiency type B. One on them had exon skipping on cDNA analysis, and a homozygous mutation was identified, located in a potential branch point sequence [12].Another patient had a homozygous missense mutation in exon 16, c.2606G>A (p.Gly869Asp), near to the one we found in our patient (c.2602G>A) [12]. Other cases were reported with homozygous PC mutations, one of them was a familial case (two brothers) of type B with compound heterozygote mutation in PC gene c.[2493_2494delGT]+[2473+2_2473+5delTGCA] [11]. This familial case had a compound heterozygous mutation like our family with PC deficiency type B.

Wang reported mosaicism in five cases and four of them had prolonged survival. These patients had very low amounts of fibroblast PC protein of 2% and 3% [9]. The authors suggested that prolonged survival is probably determined by mosaicism, presence of mRNA and residual enzyme activity [9]. The clinical expression is probably influenced by the distribution of the mutation in the developing organism. Death in the last case was because of unrelated medical complications. The type B phenotype is more associated with complex missense mutations, splice donor site mutations and deletions [9]. The amount of PC protein and residual tissue enzyme activity had an important influence on the severity of clinical phenotype of PC deficiency [7, 8].

Some genotype phenotype correlations have been proposed. Patients with type A, mostly have missense mutations, whereas patients with type B have at least one truncating mutation, in association with either another truncating mutation or a missense mutation [6, 11]. These data have a practical implication for molecular diagnosis of PC deficiency.

Treatment of PC deficiency varied between patients. Some patients have good response to biotin treatment, probably because they may carry mutations affecting biotinylation. Nevertheless , most of patients do not respond to the treatment [13]. Treatment of patients with type B with high doses of citrate or aspartate may regenerate oxaloacetate and consequently decrease metabolic disturbances, but it cannot enhance the psychomotor delay [14]. Treatment consists on stimulating residual PC enzyme activity. Correction of the biochemical disturbance can reverse some symptoms, but central nervous system damage didn't change [14]. Giving Triheptanoin to patients, provides a source for acetyl-CoA and anaplerotic propionylCoA. It has been successful for a patient with biotin-unresponsive PC deficiency type B. The patient had reversal of his hepatic failure and full correction of his biochemical abnormalities [15]. This therapeutic approach showed improvement of brain metabolism. Unfortunately, our patient died at 6 days, and was not able to have the appropriate treatment.

## CONCLUSION

Next Generation Sequencing (NGS) has increased sequencing capacity and lowered cost of sequencing, allowing detection of mutations causing genetic diseases. It is a powerful alternative to Sanger sequencing for diseases with clinical variability and genetic heterogeneity like metabolic disorder. Here, we used Clinical Exome Sequencing for a post-mortem diagnosis in a family with two deceased children with probably metabolic disorder. We illustrated the use of exome sequencing as a systematic and unbiased diagnostic tool in a pediatric case with metabolic disorder, for an appropriate management of patients and genetic counseling of their families.

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**Competing Interests**

The authors declare no competing interest.

**Authors' Contributions**

All authors read and approved the final version of this manuscript and equally contributed to its content.

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