PRENATAL DIAGNOSIS IN MITOCHONDRIAL DISEASES -CASE REPORT-

ANDREEA M. SERBAN[1], LAURENTIU CAMIL BOHILTEA[1], CRISTIAN R. STRUNGARU[1], RUXANDRA CRETU[1], DANIELA NEAGOS[1]

Key words: Mitochondrial disease, Prenatal diagnosis, HIV, Zidovudine, Genetic therapy
Abstract: Lactic acidosis, hypertrophic cardiomyopathy, muscle weakness and central nervous system impairment could rise suspicion of a mitochondrial disease. Its clinical complexity makes diagnostic process often difficult. While the gravity can range from mild to life-threatening, prenatal testing remains a valid option only in families with index cases.

INTRODUCTION

Mitochondrial disorders, one of the most recently open chapters in research, has its basis in a very polymorphic pathology which can potentially affect any organ, possible tissue-specific or multisystemic, static or progressive, having an early- or late-onset (Dimauro S., et al, 2009). Its complexity resides in different clinical patterns that can be caused by a single mutation and in a strong resemblance of signs and symptoms in some patients presenting different genetic abnormalities. The reported estimated prevalence of 9.18 in 100.000 persons (Schaefer AM., et al, 2008) takes into consideration only mitochondrial DNA mutations and does not include anomalies in nuclear genes coding mitochondrial proteins. Although considered as one of the most frequent neuromuscular inherited disease, prenatal diagnosis for known mutations already identified in a index case, remains a controversial issue.

CASE REPORT

This article presents the case of a boy apparently healthy at birth, who received a nucleoside reverse transcriptase inhibitor treatment in prevention of the materno-fetal HIV transmission. Zidovudine was administrated to the mother during pregnancy and also to the patient in the first six weeks of life. The family did not have a history of genetic diseases, that being the reason that prenatal diagnosis wasn’t performed. During the first months of life, the boy presented repeated episodes of bronchitis, a mild hypotony beginning at 7 months old and a pyelonephritis a month later associating febrile convulsions. At the age of 18 months, the patient suffered an episode of acute cardiac insufficiency and was diagnosed with hypertrophic cardiomyopathy. Laboratory exams showed a persistent lactic acidosis, with values of approximately 5 mmol/l, persistence of ketonic bodies pre- and postprandial and lactate/pyruvate rates of approximately 20. A secondary mitochondrial dysfunction caused by the Zidovudine was considered to be the cause (Finsterer J., et al, 2010). The evolution improved and the patient began walking at the age of 22 months. Nevertheless, he presented a persistent axial hypotony, frequent falls and Gowers sign. Cardiac problems persisted and the patient received an orthotopic heart transplant at 9 years with excellent tolerance.

Further analyses were performed. The muscle biopsy had a typical aspect for a mitochondrial disease with ragged-red fibres and abnormal dimensions of the organelles and the crista (Hoppel CL., et al, 2009). The analyses of enzyme levels used biopsies of striated muscle and heart and showed a severe complex I deficiency. A Blue-Native PAGE examination using fibroblasts extracted proteins from mitochondria and proved the same qualitative and quantitative abnormality of complex I (Figure 1).

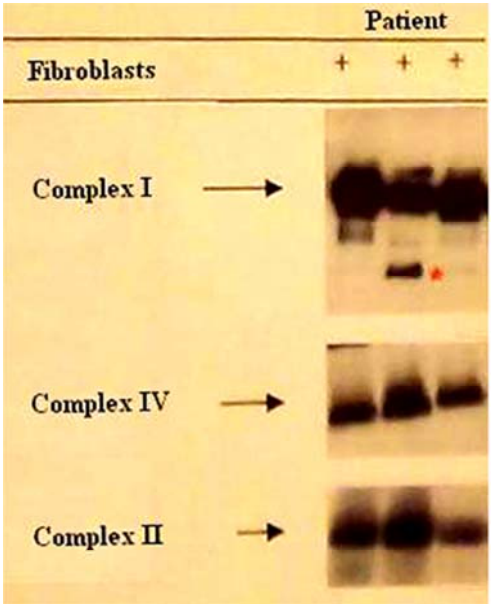

Figure 1. Blue-Native PAGE analysis of mitochondrial proteins. Middle- Patient, left, right- Controls. *Abnormal band corresponding to complex I. Normal levels of proteins of complexes II and IV in fibroblasts.

The molecular exam of the mitochondrial DNA used nucleic acid from the heart of the patient and from the mother and showed the absence of a homoplasmic or a heteroplasmic abnormality. Mutations in the nuclear genes coding for mitochondrial proteins were searched. Results for NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFA1, NDUFA2, NDUFA8, NDUFA11, NDUFB11, NDUFV1, NDUFV2 and NDUFV3 were negative, but two point mutations were identified in the ACAD9 gene (Figure 2 and Figure 3).

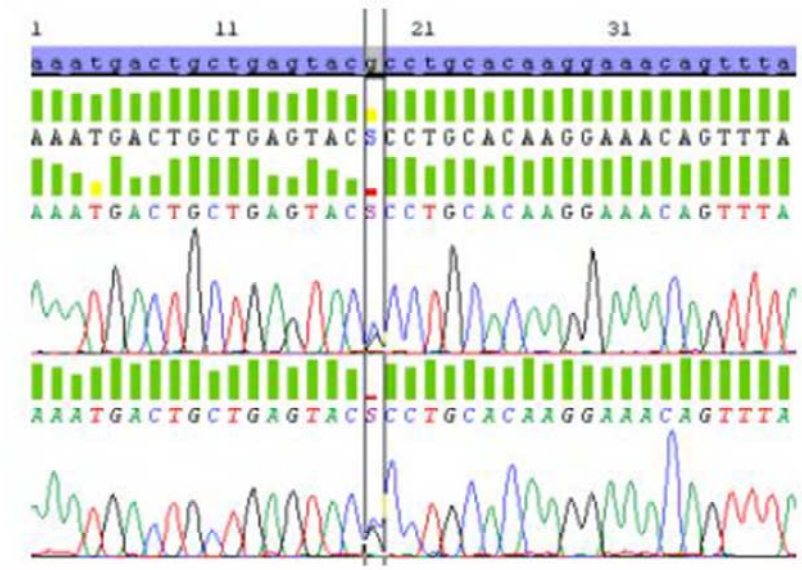

Figure 2. Heterozygous substitution c.976G>C in exon 10 determining a base replacement in the protein structure p.Ala326Pro- predicted as DAMAGING by SIFT with a score of 0.05.

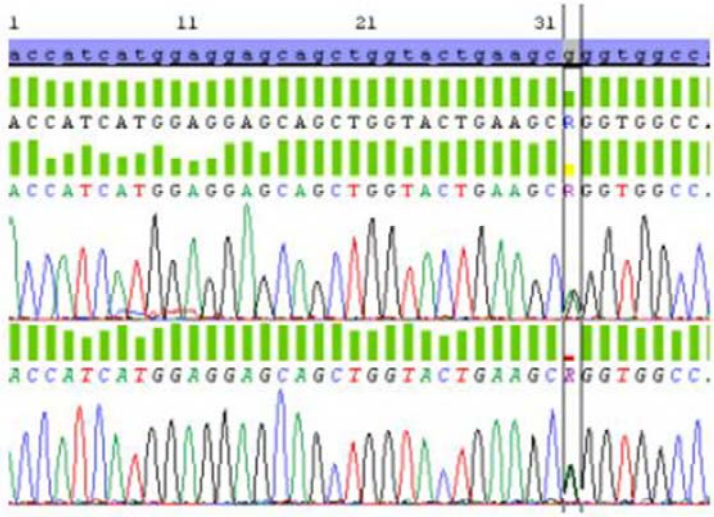

Figure 3. Heterozygous substitution c.1595G>A in exon 16 determining a base replacement in the protein structure p.Arg532Gln- predicted as DAMAGING by SIFT[5] with a score of 0.02.

Both mutations were also found in the DNA of the parents. Previously known as a catalysing protein of the rate-limiting step in the beta-oxidation of fatty acyl-CoA, ACAD9 protein is associated with NDUFAF1 and ECSIT (Nouws J. et al, 2010) and it plays the role of a

chaperone needed in the respiratory chain for the assembly and stability of complex I (Gerards M., et al, 2011; Haack TB., et al, 2012).

DISCUSSION

This case underlines the potential severity of a genetic pathology for which prenatal testing was not indicated in absence of any suspicion sign. For families with no index cases only echographic findings can be used in the diagnostic process. When present, ascites or anasarca as signs of hepatic, renal or cardiac dysfunctions, but also an abnormal fetal echocardiogram could rise the suspicion of a mitochondrial disease. Suitable only for families with mutations already identified, prenatal diagnostic techniques using chorionic villus sampling or amniotic fluid sampling allow prenatal diagnosis of genetic cause of this pathology (Brassier A., et al, 2013) . Even in situations with identified mutations, any prediction regarding age of onset or the gravity of symptoms could be given to the parents. In the same time, therapeutic options remain limited for patients with this pathology: genetic therapy, metabolic manipulation, small molecules targeting mitochondrial dysfunction, ketogenic diet and exercise (Koopman WJ., et al, 2012). Nevertheless, recent studies emphasize the benefits of germ-line gene transfer in the prevention of the transmission of pathogenic mitochondrial DNA mutations thus opening the way for new therapies (Samuels DC.., et al, 2013).

REFERENCES

1. **Dimauro S, Rustin P**. (2009) *A critical approach to the therapy of mitochondrial respiratory chain and oxidative phosphorylation diseases*. Biochim Biophys Acta.;1792:1159-67.
2. **Schaefer AM, McFarland R, Blakely EL, He L, Whittaker RG, Taylor RW, Chinnery PF, Turnbull DM**. (2008) *Prevalence of mitochondrial DNA disease in adults*. Ann Neurol.;63:35–39.
3. **Finsterer J, Segall L**.(2010) *Drugs interfering with mitochondrial disorders*. Drug Chem Toxicol ;33:138-51.
4. **Hoppel CL, Tandler B, Fujioka H, Riva A. (**2009) *Dynamic organization of mitochondria in human heart and in myocardial disease*. Int J Biochem Cell Biol.; 41:1949-56.
5. **Nouws, J. et al. (**2010) *Acyl-CoA dehydrogenase 9 is required for the biogenesis of oxidative phosphorylation complex I*. Cell Metab.*;*12, 283–294.
6. **Gerards M, van den Bosch BJ, Danhauser K, Serre V, van Weeghel M, Wanders RJ, Nicolaes GA, Sluiter W, Schoonderwoerd K, Scholte HR, Prokisch H, Rötig A, de Coo IF, Smeets HJ. (**2011) *Riboflavin-responsive oxidative phosphorylation complex I deficiency caused by defective ACAD9: new function for an old gene*. Brain. Jan;134(Pt1):210-9.
7. **Haack TB, Haberberger B, Frisch EM, Wieland T, Iuso A, Gorza M, Strecker V et al.** (2012) *Molecular diagnosis in mitochondrial complex I deficiency using exome sequencing.* J Med Genet. Apr;49(4):277-83.
8. **Brassier A, Ottolenghi C, Boddaert N, Sonigo P, Attié-Bitach T, Millischer-Bellaiche AE, Baujat G et al.** (2012) *Maladies héréditaires du métabolisme : signes anténatals et diagnostic biologique*. Arch Pediatr. Sep;19(9):959-69. doi: 10.1016/j.arcped.
9. **Koopman WJ, Willems PH, Smeitink JA.** (2012) *Monogenic mitochondrial disorders*. N Engl J Med. Mar 22;366(12):1132-41. doi: 10.1056/NEJMra1012478.
10. **Samuels DC, Wonnapinij P, Chinnery PF.** (2013) *Preventing the transmission of pathogenic mitochondrial DNA mutations: can we achieve long-term benefits from germ-line gene transfer*? Hum Reprod. Jan 7[epub].

Acknowledgements
The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

1 – "Carol Davila" University of Medicine and Pharmacy, Department of Genetics;

delia_neagos05@yahoo.com