

CASE REPORTS

Mitochondrial T9098C Sequence Change in the *MTATP6* Gene and Development of Severe Mitochondrial Disease After In Utero Antiretroviral Prophylaxis

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Mitochondrial toxicity is a well-recognized adverse effect of nucleoside reverse transcriptase inhibitor therapy for human immunodeficiency virus (HIV) infection. Transient lactic acidosis is often observed in children born after in utero antiretroviral prophylaxis against mother-to-child transmission of HIV. However, the extent and the mechanism of in utero adverse effects are largely unknown. We describe a 4-year-old girl who presented with manifestations of severe mitochondrial disease, specifically, developmental and growth delay, hypotonia, lactic acidosis, congenital cataracts, and pancreatitis. Her HIV-positive mother was receiving lamivudine, zidovudine, and nelfinavir mesylate during her pregnancy. The child tested HIV negative after birth. She was found to have a homoplasmic T9098C sequence change in the mitochondrial gene coding for adenosine 5'-triphosphate synthase 6 (*MTATP6*) that was previously reported as a mitochondrial polymorphism. This polymorphism is in the *MTATP6* gene-coding sequence and leads to the replacement of a nonpolar amino acid with a polar amino acid. Because of the typical clinical manifestations of mitochondrial disorder and because of the nature of the mitochondrial sequence change, the observed polymorphism likely predisposed this patient to develop severe antiretroviral-associated mitochondrial disease. Mitochondrial sequence alterations may be important factors in mitochondrial toxicity associated with antiretroviral treatment. Mitochondrial sequencing may be warranted in cases of persistent lactic acidosis after antiretroviral prophylaxis to further study this association.

Key Words: mitochondrial sequence change T9098C, *MTATP6* gene, mitochondrial disease, in utero antiretroviral prophylaxis, drug safety, pediatrics, pregnancy, mitochondrial toxicity.

(*Pharmacotherapy* 2009;29(12):388e–391e)

Mitochondrial toxicity associated with nucleoside reverse transcriptase inhibitors has

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Partially supported by the New York State Office of Mental Retardation and Developmental Disabilities, Albany, New York.

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been well demonstrated in both studies of mitochondrial enzyme activity and in histologic studies of mitochondrial morphology.¹ Possible mechanisms of this adverse effect are inhibition of mitochondrial DNA polymerase- γ or induction of mitochondrial mutations.²

Mitochondrial toxicity is an adverse effect of nucleoside reverse transcriptase inhibitors used as prenatal prophylaxis against mother-to-child transmission of human immunodeficiency virus (HIV). This effect was first observed in a large retrospective study of 1754 children who had been exposed to in utero antiretroviral prophylaxis.³ Eight children (0.46%) had severe

neurologic manifestations and biochemical evidence of mitochondrial dysfunction. Certain mitochondrial mutations associated with disease phenotypes were ruled out in the affected children. To our knowledge, full mitochondrial sequencing has not been carried out in patient populations such as this one.

After this initial study,³ findings from additional reports either supported or rejected this proposed, clinically significant mitochondrial toxicity due to antiretroviral exposure.⁴ Isolated cases of postnatal antiretroviral therapy-triggered mitochondrial disorders have also been reported. For instance, postnatal antiretroviral exposure was associated with late development of Leber hereditary optic neuropathy in an individual with a mitochondrial mutation who was predisposed to this disorder.⁵ In another report, mitochondrial disorder developed after antiretroviral therapy in a patient with a novel DNA polymerase- γ mutation.⁶ No clear consensus exists about the extent of such mitochondrial toxicity. However, in view of the present data, careful long-term clinical follow-up is recommended for children who are treated in utero.

We describe the case of a child who presented with manifestations of severe mitochondrial disease 4 years after in utero antiretroviral exposure.

Case Report

A 4-year-old girl was referred to our clinic because of severe mental retardation and growth delay. Her mother had been HIV-positive before her pregnancy and was receiving combination lamivudine-zidovudine, both nucleoside reverse transcriptase inhibitors, and nelfinavir mesylate, a protease inhibitor, to prevent mother-to-child transmission of HIV. The child was born at 37 weeks' gestation, with a birth weight of 2126 g. The newborn period was complicated with transient hyperparathyroidism. The infant received lamivudine, zidovudine, and nevirapine for the first month of life. She tested negative for HIV at 18 months of age.

The child's development had been severely delayed since birth. Bilateral cataracts were observed at 1 year of age and corrected surgically. Noncontrast magnetic resonance imaging of her brain was performed the same year, and the results were reported as normal. Pancreatitis was diagnosed after her third birthday and later resolved.

On follow-up visits, the last of which occurred when the patient was 6.5 years old, all growth parameters, including head circumference, were below the third percentile. The patient had generalized hypotonia and was unable to walk. She had no speech or purposeful hand movements, and she was not toilet trained. Her facial features were mildly dysmorphic, with long eyelashes and arching eyebrows.

Results of multiple genetic tests, including chromosome analysis and microarray comparative genomic hybridization analysis with a 44,000-probe system (Agilent Technologies, Inc., Santa Clara, CA), were negative. Also negative were test results for Angelman syndrome, Rett syndrome, and Smith-Lemli-Opitz syndrome. In addition, the patient tested negative for myotonic dystrophy, cystic fibrosis, galactosemia, peroxisomal disorders, and mannosidosis. Metabolic screening results were negative except for persistently elevated serum lactic acid levels. Lactic acidosis was confirmed on two separate occasions, with lactic acid levels of 26 and 28 mg/dl (normal range 4–16 mg/dl), measured at 5 and 6 years of age, respectively.

Mitochondrial DNA sequencing revealed a homoplasmic sequence change (T9098C) in the *MTATP6* gene. This alteration was previously reported as a rare polymorphism occurring in 1 of 2000 healthy individuals who were examined.⁷ The same homoplasmic sequence change was observed in the patient's mother and older brother, who both had normal cognitive development. The pregnancy with the patient's brother occurred before the patient's mother became infected with HIV.

Discussion

Our patient's clinical presentation included manifestations typically associated with mitochondrial disorder, which included persistently elevated lactic acid levels, hypotonia, early cataracts,⁸ and pancreatitis.⁹ Moreover, the mitochondrial sequence change altered a coding region of the *MTATP6* gene and replaced a nonpolar amino acid with a polar amino acid. The patient's clinical and laboratory data may indicate that the observed mitochondrial sequence change made her prone to develop severe in utero antiretroviral mitochondrial toxicity. The presence of this mitochondrial polymorphism in our patient's mother and brother indicated that it did not occur in the proband as a result of antiretroviral-associated mutagenesis. This

polymorphism did not lead to clinically significant mitochondrial disease in the patient's mother, who was receiving antiretroviral therapy. This observation suggested that it created only a predisposition to develop in utero adverse effects. Alternative explanations for the child's clinical manifestations, such as maternal drug use or an unidentified genetic disorder, could not be completely ruled out. However, multiple evaluations yielded no evidence of such etiologies.

It can be argued that the ABC efflux system would substantially decrease levels of most antiretroviral drugs in the fetus and would thus provide protection against adverse effects.¹⁰ However, lamivudine and zidovudine levels in cord blood were found to be similar to maternal levels when mothers received these drugs during pregnancy.^{11, 12}

The protein product of *MTATP6*, adenosine 5'-triphosphate (ATP) synthase 6, is a subunit of mitochondrial enzyme complex V, which has an important role in oxidative phosphorylation. Mutations of the *MTATP6* gene were previously reported in association with the syndrome of neurogenic muscle weakness, ataxia, and retinitis pigmentosa, for which typical clinical manifestations start in early childhood.^{13, 14} Severity of the phenotype of this syndrome appears to be correlated with the mutant load, where clinically significant cases are usually characterized by more than 80% mutant mitochondria.¹⁴ A common *MTATP6* mutation leading to this syndrome impairs oxidative phosphorylation and increases production of toxic oxygen radicals.¹⁵ Of interest, despite the importance of ATP synthase 6 for cell functioning, this gene has one of the highest rates of sequence variation in its coding region.¹⁶ Such variations are the result of climate-related natural selection since variants that lower coupling efficiency would reduce ATP production while increasing heat production.¹⁶

Mitochondrial toxicity has been associated with the use of nucleoside reverse transcriptase inhibitors and is caused by impaired mitochondrial oxidative phosphorylation.² Therefore, it is logical to speculate that under physiologic conditions, the polymorphism reported in our patient may only slightly decrease the efficiency of ATP production and not substantially impair cellular viability. However, if the efficiency of energy production is further impaired because of toxicity due to nucleoside reverse transcriptase inhibition, the resulting damage may become clinically significant.

Conclusion

This case report suggests that severe antiretroviral-associated mitochondrial toxicity occurred in an individual with a homoplasmic mitochondrial sequence change, which is considered to be a benign polymorphism. This case may demonstrate an important mechanism for antiretroviral-associated mitochondrial toxicity. Mitochondrial sequencing may be warranted in cases of persistent lactic acidosis after antiretroviral prophylaxis to further study this association.

Acknowledgments

We would like to thank Dr. Ed Jenkins for his constructive comments during manuscript preparation and Ava Dennie, R.P.A., for her help in the clinical care of this patient and her family.

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