A case of allelic dropout in SLC25A13 gene in a patient with Citrin Deficiency

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Abstract

Citrin deficiency is an autosomal recessive disorder caused by biallelic pathogenic variants in SLC25A13 gene. PCR and Sanger sequencing of the SLC25A13 gene is the most reliable method for definitive diagnosis of citrin deficiency. However, an incidental finding of a case with allelic dropout; a phenomenon which does occur occasionally was identified recently. Allele dropout is an amplification failure of one of two alleles caused either due to allele-specific sequence variations where primer cannot hybridize to sequence binding sites or preferential amplification of an alternate allele. This study described a case of allelic dropout in a citrin deficiency patient and strategies used to detect and to solve the case. One-month-old male infant presented with neonatal intrahepatic cholestasis was referred to Molecular Diagnostics & Protein Unit, Institute for Medical Research for SLC25A13 gene mutation analysis. The diagnostic strategy is by using long-range PCR to screen for a recurrences IVS16ins3kb. Next, PCR and sequencing of all the 18 exons were performed using specific primers. Parental samples were obtained and tested for the origin of the mutations identified in the proband. Long-range PCR showed that the proband was heterozygous for IVS16ins3kb mutation and parental testing demonstrated that the allele was maternally-inherited. Sequencing analysis to search for a second mutation discovered 23bp duplication in exon 16, as homozygous in the proband and heterozygous only in the father; suggesting allelic dropout in the proband. A PCR and sequencing with redesigned primers at exon 16 revealed a heterozygous c.1638_1660dup, p.(Ala554Glyfs*17) in both of them concluded that allele from the maternal was dropped by the original primer. No sequence variations at maternal allele that inhibit primer to hybridize, nevertheless it was the 3kb insertion in the maternal allele that caused the amplification failure with PCR and resulted in erroneous sequencing readout of a homozygous genotype. The case was successfully identified and an allelic dropout issue in SLC25A13 gene using specific primers avoiding the 3kb insertion was solved. As a conclusion, analysis of parents’ sample is important to confirm the mutation and to troubleshoot obscure findings in the proband that may lead to misdiagnosis.

Keywords: Allele dropout; SLC25A13 gene; Citrin Deficiency

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