Title: THE ROLE OF MAT1A MUTATION IN BILIARY ATRESIA DISEASE INITIATION AND PROGRESSION: AN IPSC STUDY

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Abstract:
The pathobiological mechanisms underlying disease initiation and progression in biliary atresia (BA) remain unclear. Limited studies have addressed whether liver deterioration despite surgical treatment represent a primary event affecting hepatocytes or reactive changes secondary to bile duct injuries.

A heterozygous de novo G to A mutation in the exon 8 of the MAT1A gene in a BA patient's peripheral blood DNA was identified. This patient has a poor response to Kasai portoenterostomy, requiring liver transplantation shortly after surgery. This G>A mutation created a new splice acceptor site within the exon 8. The aberrant splicing from exon 7 to this new acceptor site within exon 8 generated a mutant transcript with part of the exon 8 deletion, a frameshift leading to a new stretch of 14 amino acids and a stop codon. *MAT1A* encodes two liver-specific methionine adenosyltransferase isozymes (MATI/III), and the mutant enzymes retained only 14.4% of the wild type enzyme activity. iPSC (BA638C) was generated from this patient's blood and was differentiated into hepatocytes. Morphological/flow cytometric/RT-qPCR analysis was performed to address if MAT1A deficiency impaired hepatocyte development/functioning. Control iPSC (HKUPS-001) derived from a normal person was included for comparison. At iPSC stage, BA638C's proliferation rate was much lower than that of HKUPS-001. After hepatocyte induction, both HKUPS-001 and BA638C iPSCs developed into hepatocyte-like cells showing typical hepatocyte morphology on day 20, expressing high level of hepatic markers. However, by day 22, BA638C-derived hepatocytes gradually lost the hepatocyte features, developed into flat and spindle-shape fibroblast-like cells. Furthermore, BA638C-derived cells expressed not only low level of hepatic makers, but also elevated levels of the epithelial-mesenchymal-transition (EMT) related markers (SMA and FSP1).

In conclusion, MAT1A mutation resulted in decreased activity of MATI/III. The disrupted methionine metabolism could lead to reduced iPSC proliferation, a premature loss of differentiated hepatocyte morphology and function, and abnormal mesenchymal transformation, contributing respectively to disease initiation and progression of BA.

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