A NOVEL DEEP INTRONIC *CPS1* VARIANT CAUSING PSEUDO-EXON INSERTION: DIAGNOSTIC CHALLENGES IN UREA CYCLE DISORDERS

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Background: Diagnosing proximal urea cycle disorders (UCDs) remains challenging due to the absence of definitive diagnostic biochemical markers, which limits their inclusion in routine newborn screening programs. Although molecular genetic testing has improved diagnostic accuracy, some patients, including those suspected based on findings such as intermittently low citrulline, are found to have only a single pathogenic variant or no identifiable variants in known disease-associated genes. This raises the possibility that non-coding variants, deep intronic changes, or structural alterations may contribute to disease but remain undetected by conventional sequencing methods. Here, we report an adult male with a history of hyperammonemic encephalopathy who remained undiagnosed despite strong clinical suspicion of a UCD.

Methods: Target gene panel analysis for UCDs, including *OTC*, *NAGS*, *CPS1*, *SLC25A15*, *ARG1*, *ASS1*, and *ASL*, was performed using genomic DNA extracted from the peripheral blood. To investigate potential splicing abnormalities, reverse transcription PCR was conducted using blood-derived cDNA to amplify the full-length *CPS1* transcript.

Results: Review of the medical records revealed that this 28-year-old Japanese male developed hyperammonemic encephalopathy at 14 months of age, with a peak blood ammonia level of 237 μg/dL. Since that episode, he has been managed with a protein-restricted diet (currently 0.4 g/kg/day) and sodium benzoate, with occasional mild hyperammonemia (40–70 μg/dL). He exhibits mild intellectual impairment with an IQ of 79. Recent biochemical testing showed low blood urea nitrogen (4 mg/dL), low plasma citrulline (6.7 μmol/L), and elevated plasma alanine (1132 μmol/L). Targeted gene panel testing identified a heterozygous *CPS1* missense variant, c.840C>G, p.Lys280Asn, in exon 8, which has been previously reported in three Japanese patients with CPS1 deficiency. Subsequent mRNA analysis revealed predominant expression of an aberrant transcript containing a 121 bp pseudo-exon inserted between exons 3 and 4. Further

genomic analysis identified a deep intronic variant, c.381+178A>C, located 15 bp upstream of the novel acceptor site within the pseudo-exon, suggesting its causal role in aberrant splicing.

Conclusions: We identified a novel deep intronic *CPS1* variant causing pseudo-exon insertion in an adult with CPS1 deficiency. As most previously reported *CPS1* variants are located within coding regions, this represents a rare finding. This finding highlights the need for expanded genetic analysis, incorporating intronic regions in genetic testing to achieve accurate molecular diagnosis in UCD.