Identification of novel deep intronic PAH gene variants in patients with phenylketonuria

Xiaohua Jin[1], Yousheng Yan[2], Chuan Zhang[3], Ya Tai[4], Lisha An[1], Xinyou Yu[5], Linlin Zhang[6], Xiaofang Cao[7], Shengju Hao[3], Chenghong Yin[2], and Xu MA[8]

[1]National Research Institute for Family Planning
[2]Capital Medical University Beijing Obstetrics and Gynecology Hospital
[3]Gansu Provincial Maternity and Child-Care Hospital
[4]Peking University International Hospital
[5]General Hospital of Ningxia Medical University
[6]The Third Affiliated Hospital of Zhengzhou University
[7]National Research Institute for Family Planning Center for Genetics
[8]National Research Institute for Family Planning, Peking Union Medical College

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Abstract

Phenylketonuria (PKU) is caused by phenylalanine hydroxylase (PAH) gene variants. Previously, 94.21% of variants were identified using Sanger sequencing and multiplex ligation-dependent probe amplification. To investigate the remaining variants, whole-genome sequencing (WGS) was performed in four patients with PKU with unknown genotype to identify deep intronic or structural variants. Three novel heterozygous variants (c.706+368T>C; c.1065+241C>A; and c.1199+502A>T) were identified in a deep PAH gene intron. The c.1199+502A>T variant was detected in 60% (6/10) PKU patients. In silico prediction showed that the three deep variants may impact splice site selection and result in inclusion of a pseudo-exon. The c.1199+502A>T PAH minigene and reverse transcription PCR of blood RNA in a patient with PKU and compound heterozygous variants (c.1199+502A>T/ c.1199G>A) confirmed that the c.1199+502A>T variant creates a novel branch point and leads to the inclusion of a 25 bp in PAH mRNA (r.1199_2000ins1199+538_1199+562). Furthermore, the c.1199G>A mutation leads to the retention of an additional 17 nt in the PAH mRNA transcript (r.1199_2000ins1199+1_1199+17). These results expand the PAH genotypic spectrum and highlight that deep intronic analysis of PAH can improve genetic diagnosis in undiagnostic patients.

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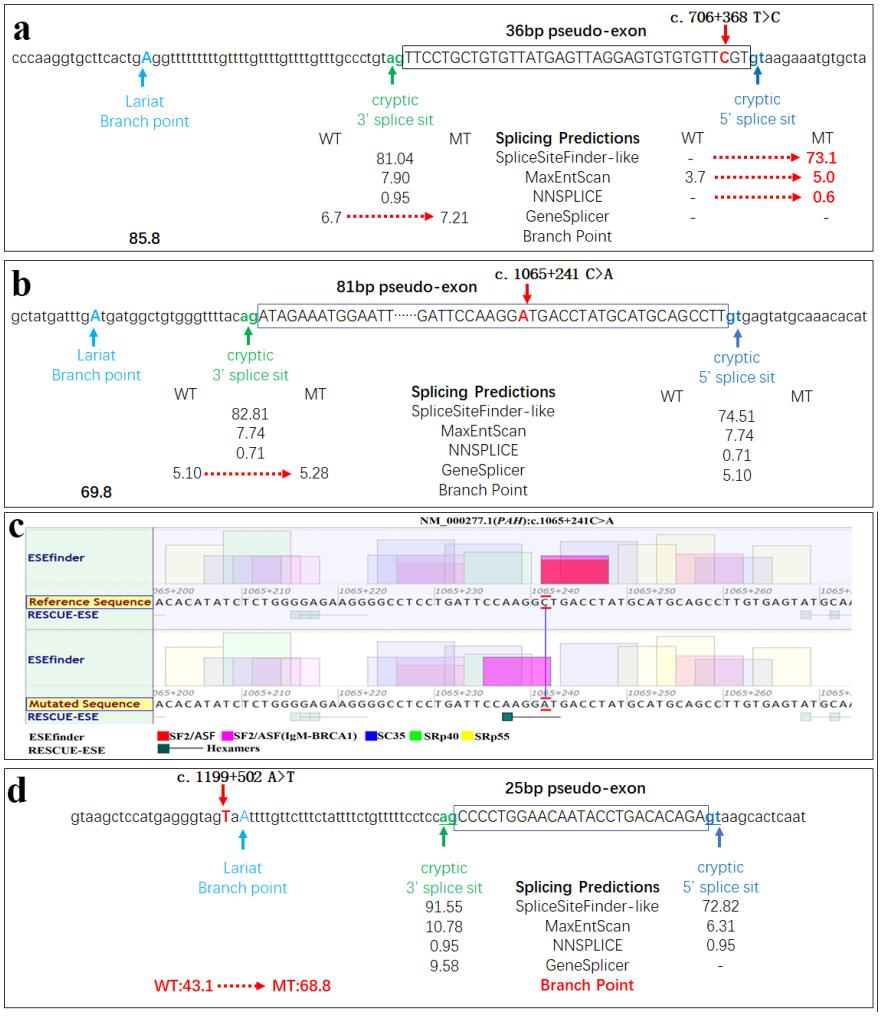

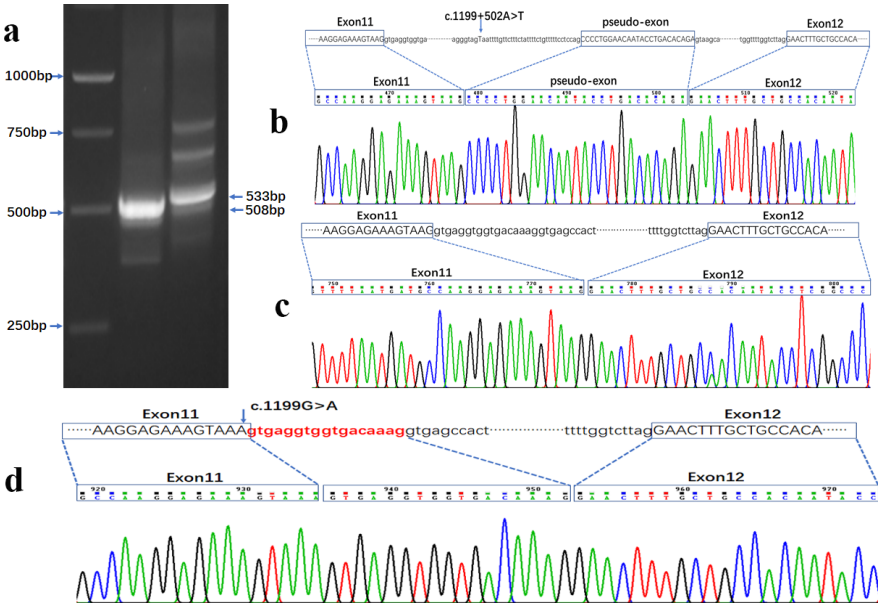

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