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Pathogenetic Mechanisms of PTS Mutations Associated with Mild Clinical Phenotype

Yen-Hui Chiu[1], Ying-Chen Chang[1], Kwang-Jen Hsiao[2], Tze-Tze Liu[1]

[1]Department of Education and Research, Taipei City Hospital, Taipei, Taiwan, National Yang-Ming University, Taipei, Taiwan, Taipei, Taiwan, [2]Preventive Medicine Foundations, Taipei, Taiwan

Objective: Phenylketonuria (PKU) and hyperphenylalaninemia (HPA) may be caused by deficiency of phenylalanine-4-hydroxylase or its important cofactor, tetradhydrobiopterin (BH4). BH4 is also involved in nitric oxide synthases, tyrosine hydroxylation and tryptophan hydroxylase, the latter two are crucial enzymes for the biosynthesis of neurotransmitters dopamine and serotonin, respectively. BH4 deficient patients without early diagnosis and proper treatment show impairment in phenylalanine catabolism accompanying with a deficiency in neurotransmitters. Depletions in any of the enzymes involved in BH4 biosynthesis or regeneration leads to BH4 deficiency. Among these, 6-pyruvoyl-tetrahydropterin synthase (PTPS, gene symbol: PTS) deficiency (MIM 261640) is the most common form of BH4 deficiency. To date, 36 mutations, including 27 missense, 2 nonsense, 1 small deletion, 1 frameshift, 4 splicing and 1 initiation mutations, were identified in PTS gene in Chinese populations. The c.166G>A and c.84-291A>G mutations were found to associate with mild clinical phenotype of PTPS deficiency. Here we investigated the pathogenesis mechanism of the two mutations.

Methods: Wild type PTS cDNA was first cloned into vector pcDNA3. The missense mutant alleles were induced into the plasmid by site direct mutagenesis. The plasmid carrying wild type and/or mutant PTPS were transfected into COS-1 cells followed by analyzing PTPS enzyme activity in lysate. On the other hand, the cDNA of cells carrying the intronic mutation were isolated, amplified and subcloned into the vector followed by sequencing analysis.

Results: The COS-1 cells overexpressing c.166G>A mutant protein presented normal PTPS enzyme activity (106.53 $\pm$22.3% of that in cells transfecting wild-type plasmid). However, the activity of PTPS was dramatically deceased to approximately 10% when c.166G>A were cotransfected with other mutant alleles while no enzyme activity reduction was observed in cells cotransfected with plasmid carrying c.166G>A and wild type PTS cDNA. ESE finder predicted an alteration of the SRp40 splicing protein binding site when the c.84-291A>G mutation is present. A 79bp pseudoexon flanked by consensus splice site can only be identified in cells carrying the c.84-291A>G mutation but not in the control cells. Furthermore, in subcloning of PTS cDNA from the skin fibroblasts of a patient heterozygous for the c.259C>T and c.84-291A>G mutations, 55% of clones represented the c.259C>T mutation or exon 3 skipping. Intriguingly, only 9% of the clones contained the 79bp pseudoexon, whilst the other 37% of clones represented a normal PTS cDNA.

Conclusions: We speculate that the c.166G>A may be susceptible to other mutant PTPS protein but still retained low PTPS enzyme activity. On the other hand, the c.84-291A>G mutation may only create a weak binding site for SR splicing factors and allow a significant portion of normal transcript to be formed, resulting in a mild form hyperphenylalninemia.

Contact Information: Yen-Hui Chiu (yhchiu@ntu.edu.tw)