Abstract: Two mutations in the cystathionine $\beta$-synthase (CBS) gene were found in two Japanese siblings with pyridoxine non-responsive homocystinuria who had different methionine levels in their blood during the neonatal period. Both patients were compound heterozygotes of two mutant alleles: one had an A-to-G transition at nucleotide 194 (A194 G) that caused a histidine-to-arginine substitution at position 65 of the protein (H65R), while the other had a G-to-A transition at nucleotide 346 (G346A) which resulted in a glycine-to-arginine substitution at position 116 of the protein (G116R). The two mutant proteins were separately expressed in Escherichia coli, and they completely lacked catalytic activity.

Despite their identical genotypes and almost equal protein intake, these siblings showed different levels of blood methionine during the neonatal period, suggesting that the level of methionine in blood is determined not only by the defect in the CBS gene and protein intake, but also by the activity of other enzymes involved in methionine and homocysteine metabolism, especially during the neonatal period. Therefore, high-risk newborns who have siblings with homocystinuria, even if the level of methionine in their blood is normal in a neonatal mass screening, should be followed up and diagnosed by an assay of enzyme activity or a gene analysis so that treatment can be begun as soon as possible to prevent the development of clinical symptoms. In addition, a new, more sensitive method for the mass screening of CBS deficiency in neonates should be developed.


Keywords: cystathionine $\beta$-Synthase Deficiency, homocystinuria, mutation analysis, neonatal mass screening
Patients

RNA, DNA and cDNA Preparation

Amplification of cDNA, subcloning and sequencing

Detection of mutations in genomic DNA

Expression of CBS protein in E. coli
Detection of mutation

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Enzyme activities of the wild-type and mutant CBS in E. coli

(A) A194G

(B) G346A

The Journal of Medical Investigation Vol.46 1999
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