Mutation Report

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Novel Mutations (H337R and 238–362del) in the *CPS1* Gene Cause Carbamoyl Phosphate Synthetase I Deficiency

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Key Words
Mutation · Carbamoyl phosphate synthetase I deficiency · Hyperammonemia · Urea cycle

Description of the Mutation

One mutation is an A to G transition at nucleotide 1010 of the *CPS1* gene (GenBank accession No. Y15793), which leads to an amino acid change from histidine to arginine at codon 337. Another mutation in the other allele is a 4.2-kb deletion at nucleotide 117646–113441 (GenBank accession No. AC008172), which leads to a 375-bp inframe deletion at codon 238–362 (three-exon skipping).

Source of Material

Carbamoyl phosphate synthetase I (CPS1) deficiency (MIM 237300) is an autosomal recessive disorder caused by the deficient activity of CPS1 (EC 6.3.4.16), affecting the first enzyme step in the urea cycle. A Japanese girl, the first child of non-consanguineous parents, was delivered at term with low birth weight (2,394 g). At day 9 of life, she showed lethargy and grunting with severe hyperammonemia (1,550 µg/dl). She was suspected to suffer from CPS1 deficiency because of elevated blood glutamine and glutamic acid concentration, low blood citrulline concentration and absence of the orotic aciduria. By intensive care including mechanical ventilation, exchange blood transfusion and peritoneal dialysis, she gradually recovered with a decrease in the blood ammonia level, and was discharged at day 68 of life. After discharge, she continued to receive several medications including oral *l*-arginine, sodium benzoate and *l*-carnitine, and a low-protein diet. At 16 months of age, we performed an open biopsy from the liver, and demonstrated that CPS1 activity of the liver specimen was below the detectable level, while the activities of ornithine transcarbamylase, arginosuccinate synthetase, argininosuccinate lyase and arginase were within normal limits. She was diagnosed enzymatically as having CPS1 deficiency. Although we measured the blood levels of ammonia every 1–2 months and confirmed them to be around the normal range, she has failed

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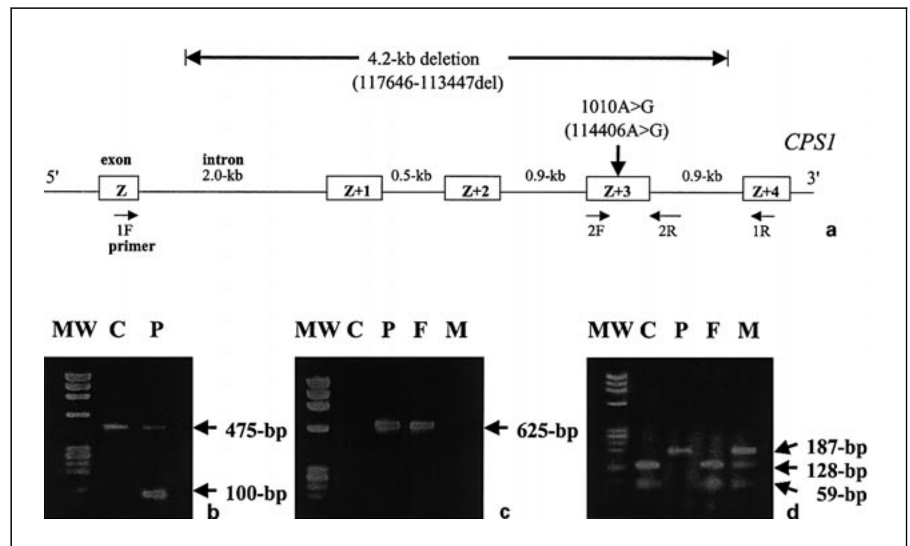

Fig. 1. a Schematic representation showing a 4.2-kb deletion mutation, an 1010A → G (114406A → G) mutation and placement of PCR primers in the *CPS1* gene. **b** An agarose gel electrophoresis of the RT-PCR fragment amplified using primers, 1F and 1R. The fragments from the patient showed both a normal 475-bp and an aberrant short 100-bp band. **c** An agarose gel electrophoresis of PCR fragments amplified with the genomic DNAs using 1F and 1R. Aberrant short 625-bp bands were amplified with the patient's and her father's samples. The normal 4.8-kb band was not amplified. **d** An agarose gel electrophoresis of the *Nla*III-digested PCR fragments amplified with the genomic DNAs using 2F and 2R. 187-bp fragments with a control's and her father's samples were digested to 128-bp and 59-bp bands. Her mother showed a heterozygous pattern. The patient showed a homozygous mutation pattern. 1F: 5′-ACCCCACAAAAGTGGTAGCTGTAG-3′, 1R: 5′-ACAGCGAAGAAGGGTTTGCT-3′, 2F; 5′-GGCAGAATCAGCCTGTTTTG-3′, 2R: 5′-AGCCCTGCGTTTCTCATCAC-3′. MW = ΦX174/*Hae*III digest; C = normal control; P = patient; F = patient's father; M = patient's mother.

to thrive. At 27 months of age, her weight was 6.8 kg (–3.3 standard deviations from normal mean value for age). She showed poor interest in her surroundings and was confined to bed.

Methods

After extracting total RNA from the liver, we analyzed the *CPS1* gene by reverse transcription PCR. We synthesized 10 pairs of primer sets to cover the entire coding region (4.5 kb) and performed heteroduplex analysis with MDE gel (FMC), adding an equal amount of control samples to the patient samples. The aberrant bands detected were subcloned into pGEM-T easy plasmid (Promega) and sequenced. The mutation seen in the cDNA was also confirmed by genomic sequencing or PCR restriction fragment length polymorphism analysis.

Evidence for Disease Association

Our patient was demonstrated to be a compound heterozygote with a 4.2-kb deletion at nucleotide 117646–113441 and 1010A → G (114406A → G) in the *CPS1* gene (fig. 1a). The deletion mutation was demonstrated to lead to 375-bp in frame deletion (three-exon skipping) in mRNA (fig. 1b), which was suspected to cause a loss of the CPS1 activity. Her father carried this deletion mutation (fig. 1c). Since the 1010A → G mutation leads to loss of *Nla*III restriction site, we performed a PCR digestion method (fig. 1d). Her mother showed a heterozygous pattern. The patient showed a homozygous mutation pattern. This is because the PCR site was lost in the other allele as a result of the gross deletion. The 1010A → G mutation was not seen in 206 alleles of normal Japanese controls. We also found that His-337 is evolutionarily highly conserved at the corresponding position in CPS1. Miran et al. [1] investigated the role of the conserved histidine residues in the amidotransferase catalytic domain of *Escherichia coli* CPS, one of which was His-312 corresponding to His-337 of human CPS1. This indicated that His-312 plays a critical role in binding glutamine to the active site in *E. coli* CPS. However, human CPS1 does not process glutamine [2], suggesting that the exact mechanism of dysfunction of human CPS1 caused by the H337R mutation

remains unclear. The other mechanisms induced by this mutation may also be possible, such as decreased expression level of messages and instability of CPS1 protein product, which result in a significant loss of CPS1 activity. In conclusion, our results indicate the H337R and 238–362del are novel mutations responsible for the CPS1 deficiency.

Acknowledgments

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References

1 Miran SG, Chang SH, Raushel FM: Role of the four conserved histidine residues in the amidotransferase domain of carbamoyl phosphate synthetase. Biochemistry 1991;30:7901–7907.
2 Rubio V: Structure-function studies in carbamoyl phosphate synthetases. Biochem Soc Trans 1993;21:198–202.

Announcement

Third Annual Clinical Cancer Genetics

A Practical Application/Case-Based Approach

Saturday, September 15, 2001
Jointly Sponsored by the Johns Hopkins
University School of Medicine and
The Mid Atlantic Cancer Genetics Network

Location: Wyndham Baltimore Inner Harbor, Baltimore, Maryland.

Course description: This one-day symposium will provide state-of-the-science information on clinical cancer genetics, gene testing and relevant clinical management strategies. Breast/ovarian and colon cancer are targeted because there exist clear indications for gene testing, screening and surveillance interventions. Genetic information concerning melanoma and pancreatic cancer is quickly evolving. Experts in the field of cancer genetics who grapple with the day-to-day issues and concerns of individuals and families at increased risk for cancer due to an inherited predisposition will provide tangible strategies and recommendations for risk assessment, counseling, and surveillance. A case analysis approach will be employed. Current legal issues will be discussed with attention to implications for the practitioner. The goal is to help the clinician respond in an informative manner that addresses fully patients' concerns and questions.

Target audience: This program is targeted for physicians (including internists, family practitioners, gastroenterologists, oncologists, gynecologists), nurses, genetic counselors, and other interested health care providers.

Accreditation statement: This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of The Johns Hopkins University School of Medicine and Mid Atlantic Cancer Genetics Network (MACGN). The Johns Hopkins University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians and takes responsibility for the content, quality and scientific integrity of this CME activity.

Credit designation statement: The Johns Hopkins University designates this educational activity for a maximum of 6 hours in category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent in the educational activity.

Other appropriate credit is pending.

Fees: Physicians – $150; MACGN affiliated physicians – $100; residents, fellows, genetic counselors, nurses and other health professionals – $100; students – $75.

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