

# A Novel Human Catalase Mutation (358 T-del) Causing Japanese-type Acatalasemia

Submitted on 12/15/95

(communicated by Ernest Beutler, M.D., 12/15/95)

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**ABSTRACT:** Japanese-type acatalasemia is characterized by the almost total loss of catalase activity in red cells and is often associated with ulcerating oral lesions. A splicing mutation in intron 4 of the catalase gene has so far been a sole disease-causing mutation found in Japanese-type acatalasemic patients. We report here a novel single base deletion in the catalase gene causing Japanese-type acatalasemia. The patient was a 72-year-old Japanese male. His maternal grandmother and his father were first cousins. Molecular analysis using non-RI PCR-SSCP analysis combined with direct sequencing revealed a deletion of the 358th thymine in exon 4 of the patient's catalase gene. The proband was a homozygote and his mother and his three children were heterozygotes for this mutation. The frame shift caused by the nucleotide deletion should alter the downstream amino acid sequence and introduce a new termination codon TGA 43 bp 3' to the mutation. Although the truncated peptide chain consisted of 133 amino acid residues might be translated in the patient's tissue, such an aberrant protein is expected to be extremely unstable and have no catalytic function at all. Our results suggest that Japanese-type acatalasemia is heterogeneous at molecular level.

**Keywords:** acatalasemia, Japanese-type, catalase, deletion mutation, frame shift, PCR-SSCP

## INTRODUCTION

Human catalase is a tetramer comprised of four identical subunits each of which consists of 526 amino acid residues (1). The hereditary deficiency of the enzyme (acatalasemia) is inherited as an autosomal recessive trait. Two different types of human acatalasemia can be distinguished clinically and biochemically (2). The severer form, Japanese-type acatalasemia, is characterized by the near total loss of catalase activity in red cells and is often associated with

ulcerating oral lesions. Heterozygotes of Japanese-type acatalasemia show half-normal red cell catalase activity (hypocatalasemia). The asymptomatic Swiss-type acatalasemia is characterized by the residual catalase activity with aberrant biochemical properties and the normal enzyme activities in heterozygotes. Of about 50 unrelated Japanese-type acatalasemia families, four have so far been studied molecular genetically, and the identical splicing mutation, a G to A transition at the 5th nucleotide in intron 4, was detected in all the cases (3,4). Several

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polymorphic base substitutions have also been found in the patients and normal subjects (3,4).

We report here a novel single base deletion in exon 4 of the catalase gene causing Japanese-type acatalasemia, indicating that the disease is heterogeneous at the DNA level.

## MATERIALS AND METHODS

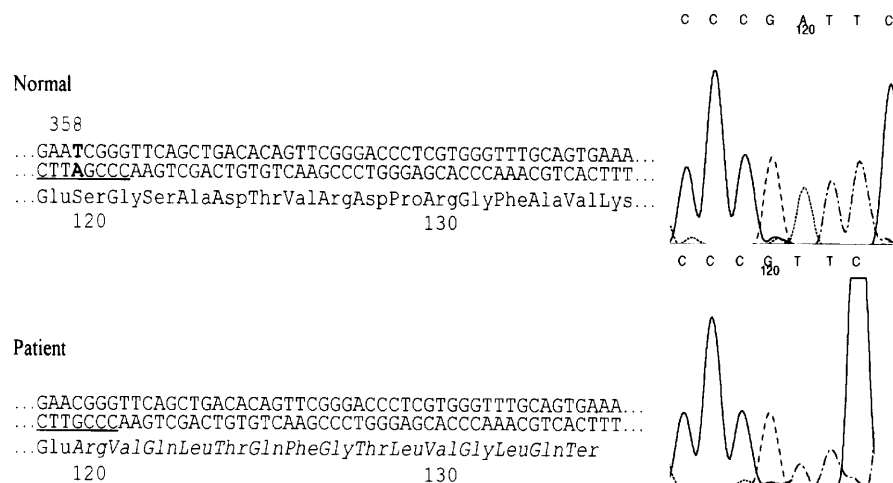
### Patient

The patient was a 72-year-old Japanese male. His maternal grandmother and his father were first cousins. There was no family history of ulcerating oral lesions. He was admitted to the Tokyo Medical College Hospital for the treatment of his laryngeal cancer. During the laryngectomy operation, operators noted that the patient's tissue turned dark brown and did not form bubbles when diluted hydrogen peroxide was applied. The blood methemoglobin level was found to be increased to 10.8%. His skin in the buccal region and oral and pharyngeal mucosa became edematous and developed bullae. The catalase assay by measuring the rate of decomposition of  $H_2O_2$  by

the enzyme (5) showed that the red cell catalase activity was deficient in the proband (<0.05% of normal) and was half-normal in his mother and all of his three children.

### Mutation Analysis

A previously described non-radioisotopic PCR-SSCP analysis (6) was used to screen the catalase gene for mutations. Genomic DNA samples were obtained from the proband, his mother and children and normal controls after informed consent. Oligonucleotide primers were synthesized according to the sequences by Kishimoto et al. (4). We used a sense primer 5'-TTCCTGTAAACTTAGTTTTTGG for exon 5 and an antisense primer 5'-TGCTGAATTAACATTAATGTAA for the third portion of exon 13 in place of their original ones. When mobility shifts were found in PCR-SSCP analysis, corresponding PCR products were directly sequenced by an automated DNA sequencer using a dideoxy fluorescent dye termination protocol (Perkin Elmer).



**Figure 1.** Partial DNA sequence of exon 4 of the normal and acatalasemia gene showing a deletion of the thymine at nucleotide 358 and predicted downstream amino acid alterations in the patient (shown in italic letters).

## RESULTS AND DISCUSSION

Direct sequencing of exon 4 that showed a clear mobility shift revealed a deletion of the thymine at nucleotide 358 in the patient's catalase gene (Fig. 1). Because the deletion destroyed a native Hinf I cleavage site, we could readily confirm the existence of the mutation. We found that the proband was a homozygote and his mother and all of his three children were heterozygotes for this mutation. The frame shift caused by the nucleotide deletion should alter the downstream amino acid sequence and introduce a new termination codon TGA 43 bp 3' to the mutation. Although the truncated peptide chain comprised of 133 amino acid residues might be translated in the patient's tissue, such an aberrant protein is expected to be extremely unstable and have no catalytic activity at all.

This is the second reported mutation causing Japanese-type acatalasemia. Only a splicing mutation in intron 4 has been reported prior to ours (3,4). The latter has been identified in four unrelated acatalasemia families and was considered to be a dominant genetic change in Japanese acatalasemic patients (4). Both of these two mutations should lead to the near total loss of enzyme protein and subjects bearing these mutations could not be distinguished by their phenotypes. Whether the present deletion mutation accounts for several unrelated cases of Japanese acatalasemia or it is merely a sporadic mutation found only in the present pedigree

remains unknown. Further molecular analysis of a larger number of acatalasemic families will provide more insight into the genetic condition of Japanese-type acatalasemia.

## ACKNOWLEDGMENTS

We thank Atsuko Sakuma for her technical assistance. This work was supported in part by research grants from the Ministry of Education, Science, Sports and Culture, and the Ministry of Health and Welfare, Japan.

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