

*Short Communication*

POLYMORPHISM OF EXTRACELLULAR SUPEROXIDE  
DISMUTASE (EC-SOD) GENE: RELATION TO  
THE MUTATION RESPONSIBLE FOR  
HIGH EC-SOD LEVEL IN SERUM

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**Summary** Extracellular superoxide dismutase (EC-SOD) with amino acid substitution R213G generated by the nucleotide substitution 760C→G in the heparin binding domain is responsible for the high EC-SOD level in serum. We identified the two DNA polymorphic sites in the coding region of EC-SOD gene related to the 760C→G and determined the allele frequencies. The polymorphisms were A and G at nucleotide position (nt.) 241 and C and T at nt. 280 near the N-terminal. The haplotype frequencies in Japanese were 241A280C: 0.45, 241G280T: 0.37, and 241G280C: 0.18. The haplotype of 241A280T did not exist. The mutation 760C→G must occur on the allele having the haplotype of 241G280T.

**Key Words** extracellular superoxide dismutase (EC-SOD), DNA polymorphism, haplotype, mutation

*Introduction*

Extracellular superoxide dismutase (EC-SOD) is an SOD isozyme mainly distributed in the extracellular fluid. It is located on the endothelial cell surface

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according to the heparin binding capacity. The EC-SOD gene is located on human chromosome 4 (Gallagher *et al.*, 1992). Its cDNA sequence was reported by Hjalmarsson *et al.* (1987), and the entire coding region was included in the same exon (Yamada *et al.*, 1995). Recently, a single point mutation of the C to G (760C→G) near the carboxyl terminal has been identified in 2% of the plasma donors in Sweden (Sandstöm *et al.*, 1994), in 3.3% in USA (Folz *et al.*, 1994), and 6% in Japan (Yamada *et al.*, 1995). The amino acid substitution (R213G) is important for the heparin binding capacity which is one of the characteristics of EC-SOD (Adachi *et al.*, 1996). In the process of the gene analysis, we found two polymorphic sites near the N-terminal of EC-SOD. Herein, we determined the frequencies of these polymorphisms, and clarified their relationship with the mutation 760C→G responsible for the high EC-SOD level in serum.

#### *Materials and Methods*

All the methods of EC-SOD gene analysis were described previously (Yamada *et al.*, 1995). DNA fragments (910 bp) including the entire coding region of EC-SOD gene were amplified using a primers pair (SE1A and SE1B) from genomic DNAs. The polymerase chain reaction (PCR) was carried out for 40 cycles (94°C, 1 min; 60°C, 1 min; 70°C, 2 min). After electrophoresis in a low melting point agarose gel (1.5%) (Ultra Pure, BRL, Gaithersburg, USA), the fragments were extracted from the gels, purified by PEG precipitation, and the nucleotide sequences were determined according to the simplified direct sequencing method using the 7-deaza-dGTP Reagent Kit for Sequenase ver. 2 (Amersham USB, Little Chalfont, England). The mutation site (760C→G) were sequenced using a primer SE2B, and two polymorphic sites using SE5B. For the cloning study, PCR products was ligated to the PCR II vector (Invitrogen, San Diego, USA), separated to the single clone and the sequence checked.

#### *Results and Discussion*

From the genomic DNAs derived from 253 Japanese healthy persons and hemodialysis patients, the coding region of EC-SOD was PCR-amplified using the specific primers. The substitutions of the A to G at nt. 241 (241A→G), and the C to T at nt. 280 (280C→T) compared with the sequences described by Hjalmarsson *et al.* (1987), were detected by the direct sequencing of the PCR products. The substitution 241A→G generates the amino acid change of Thr to Ala (T40A), but 280C→T is silent. The substitution T40A did not affect heparin binding capacity or the specific activity of EC-SOD (unpublished data). The 241G creates a new *Bss*HIII restriction site.

The subjects were classified into; 222 without the mutation 760C→G, and 29 heterozygous, and 4 homozygous for the mutation. As shown in Table 1, there were differences in frequencies of the two polymorphisms between the cases with and without the mutation 760C→G. The frequencies of 241G and 280T in the

Table 1. The allele frequencies of the DNA polymorphisms in the groups with and without the mutation 760C→G.

Allele (n)	Without 760C→G	With 760C→G	
	444	Heterozygote 58	Homozygote 8
241A	0.45	0.26	0.00
241G	0.55	0.74	1.00
280C	0.63	0.39	0.00
280T	0.37	0.61	1.00

Table 2. The haplotype frequencies of the DNA polymorphisms in the individuals without the mutation.

Haplotype	Frequency	
	Japanese	Caucasian
241A280C	0.45	0.30
241A280T	0.00	0.00
241G280C	0.18	0.55
241G280T	0.37	0.15

individuals with the mutation were significantly higher than those without the mutation. In the 4 homozygous individuals, only a haplotype 241G280T was found. These findings suggested that the mutation 760C→G was closely related to the detected polymorphisms. Furthermore, the substitution 280T may have occurred on the allele with 241G, since all individuals with 280T had 241G.

To clarify the relationship between the 760C→G and haplotypes of two polymorphic sites, we carried out cloning studies of the heterozygous individuals for both polymorphisms (241A and G, 280C and T). We analyzed the first 20 clones derived from 5 individuals with the mutation 760C→G on one allele. All 10 clones with the mutation were a haplotype of 241G280T, whereas the other 10 clones without the mutation were a haplotype of 241A280C. Analyses of 40 clones derived from 10 individuals without the 760C→G showed the following results: 10 clones with a haplotype of 241A280C, 8 clones with 241G280C, and 22 clones with 241G280T. No clone with a haplotype of 241A280T was detected in any clone with or without the 760C→G. These findings suggested the absence of an allele having the haplotype of 241A280T and the occurrence of a mutation 760C→G associated with a high EC-SOD level in serum on the allele of the haplotype of 241G280T.

The frequencies of the haplotypes in the Japanese were calculated from the data in Table 1, based on the absence of 241A180T (Table 2). The frequency in the Caucasians was also calculated from our preliminary analysis on 10 individuals without the 760C→G. In the frequency of haplotype of 241G280T on which the

760C→G seemed to have occurred, the value in the Caucasians (0.15) was significantly lower than that in the Japanese (0.37). This difference may reflect the higher incidence of the high EC-SOD levels in serum in the Japanese due to the mutation 760C→G which may have occurred on the allele of haplotype of 241G280T.

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