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Short Communication

**SAC I RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) RELATED TO THE HUMAN CST2 GENE**

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Abstract

Restriction Fragment Length Polymorphism (RFLP) related to cystatin gene (CST) family was detected in the Japanese population by using restriction enzyme Sac I. A polymorphic site, located at 0.9kb from the 3‘ end of the CST2 gene, revealed a two allele polymorphism with band sizes of 3.5kb and 8.3kb by hybridization with probe including exon 2 of the CST1 gene. The gene frequencies in the Japanese population were 0.326 for 3.5kb allele and 0.674 for 8.3kb allele (n=86). The phenotypes of the polymorphism showed no association with the previously reported electrophoretic cystatin SA protein phenotypes²².

Key words: Type 2 cystatin gene family—CST2—RFLP—SNP—Salivary cystatin SA—Polymorphism

The human type 2 cystatin gene family is a multigene family composed of seven members localized on chromosome 20p11.²²,²³,²⁴ and five of these genes (CST1-5) produce proteins which are secreted in human saliva.²³,²⁴ The presence of genetic polymorphisms in the human type 2 cystatin gene family has been demonstrated in the CST3, CST5, and CST2 genes.²³,²⁴,²⁵, and a number of single nucleotide polymorphisms (SNPs) related to the type 2 cystatin gene family have been submitted in the JSNP database (the database of Japanese single nucleotide polymorphism) with recent progress on genomic sequencing: 1 SNP for CST1, 4 for CST3, 4 for CST4, 5 for CST5. In this paper, we report a RFLP related to the CST2 gene, and also show a relationship with the previously reported cystatin SA...
protein polymorphism. After informed consent was obtained, blood and saliva samples were collected from 86 subjects, and genomic DNA was obtained from the blood by usual Phenol/Chloroform extraction method. Genomic DNA was digested with Sac I, electrophoresed on a 0.8% agarose gel, and transferred to nylon membranes (NY-2N, Schleicher & Schuell). A 0.86 kb NeoI-Neol fragment including exon 1 of the CST1 gene and a 0.6 kb EcoRI-NeoI fragment including exon 2 of the CST1 gene (Fig. 2) were used as probes for hybridization. The fragments were labeled with [32P]-dCTP by nick translation. The filters were washed twice with 3X SSC including 0.5% SDS at 68°C for one hour and once with 0.3X SSC including 0.5% SDS at 68°C for one hour. The filters were exposed two days on Kodak X-OMAT™AR radiographic film. Salivary cystatin protein polymorphism was typed as previously described.

By Southern hybridization using exon 1 and exon 2 probes, no difference was observed with the exon 1 probe, but a distinct difference was observed in 8.3 kb and 3.5 kb bands using the exon 2 probe (Fig. 1). Pedigree analyses of three families confirm that these three types follow Mendelian inheritance. Among the 86 samples, 37 (39.1 expected) possessed a 8.3 kb band (genotype 8.3/8.3), 42 (37.8 expected) possessed two bands of 8.3 and 3.5 kb (genotype 8.3/3.5), and 7 (expected 9.1) possessed a 3.5 kb band. The gene frequencies for the 8.3 kb allele and 3.5 kb allele obtained in the Japanese population were 0.674 and 0.326, respectively. There was a good agreement between observed and expected numbers of genotypes assuming a Hardy-Weinberg equilibrium ($\chi^2 = 1.06, d.f. = 1, 0.3 < p < 0.5$).

Fig. 2 shows a restriction map of type 2 cystatin gene family reported by Saitoh et al. including data by Freije et al. Among these genes, the CST2B gene had been proposed to be an allele of the CST2 gene. If the exon 2 probe hybridized to all known genes reported up to date, each gene should generate a 2.7 kb band for CST1, a 3.5 kb band for CST2, a band larger than 5.6 kb for CST2B, a 2.5 kb band for CST3, a 2.8 kb band for CST4, a band larger than 2.84 kb for CST5, a 1.95 kb band for CSTP1 and a 2.84 kb band for CSTP2. Pairwise comparisons of the nucleotide sequences between the exon 2 EcoRI/NeoI probe and six CST genes showed homologies of 100% for CST1, 93% for CST2 (or CST2B) and CST4, 68% for CST3, 65% for CST5, 61% for CSTP1, and 62% for CSTP2. From these observations, it was considered that the most intensified 2.8 kb band was a mixture derived from CST1 and CST4, and the 3.5 and 8.3 kb bands with similar intensities were derived from CST2 and CST2B, respectively, because the homology between the exon 2 probe and these three genes was high (93–100%); the 3.5 kb band matched the expected size derived from CST2, and the 8.3 kb band could only be derived from CST2B. The CST2 gene contained in the sequence from clone XXYac-60D10 on chromosome 20 (Accession no. AL591074) has two Sac I sites spanning 8.2 kb and including
exon 2 and exon 3, which coincides with CST2B and supports the present observation.

We have already reported a salivary protein polymorphism derived from the CST2 gene\(^{13,23}\). This polymorphism is caused by two point mutations in exon 2 and exon 3 of the CST2 gene. When 78 samples were compared with respect to the association of phenotypes between salivary cystatin SA protein polymorphism and Sac I RFLP, there was no significant association (Table 1). Thus these polymorphisms might have occurred independently.

It will be possible to apply the present polymorphisms of the CST2 gene to forensic studies and linkage studies on chromosome 20.

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**REFERENCES**


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**Table 1**  Relationship between Sac I RFLP genotypes and salivary cystatin SA (CST2) genotypes

<table>
<thead>
<tr>
<th>Cystatin SA (CST2) genotypes</th>
<th>Sac I RFLP genotype</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.3/8.3</td>
<td>3.5/3.5</td>
</tr>
<tr>
<td>CST2<em>1/CST2</em>1</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>CST2<em>1/CST2</em>2</td>
<td>7</td>
<td>6</td>
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<tr>
<td>Total</td>
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<td>38</td>
</tr>
</tbody>
</table>


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