

Figure 2

(1) Protocol for detection of NAT2 3SNPs by electrochemical DNA chip.

(2) PCR products obtained by the multiplex PCR procedure.

PCR products were confirmed by 4% agarose gel electrophoresis.

Lane 1: Marker pUC19/MspI (Nippon Gene, Toyama, Japan), Lane 2: PCR products

342bp: C481T target, 317bp: G590A and G857A target

(S/N) ratio was calculated from these voltammetric experiments. The signal means the I_{pa} value from hybridization on wild or mutant probes, whereas the noise means the I_{pa} value on control probe. The S/N ratios on 590-G probe and 590-A probe for target 590 G/G were 2.1 and 1.1, respectively. Similarly, S/N ratios on 590-G probe and 590-A probe for target 590A/A were 1.2 and 2.5, and for target 590 G/A were 2.0 and 2.2, respectively. Comparable results were obtained for the other SNPs. S/N ratios of perfect-match-combinations between probes and target DNAs were in the range from 2.0 to 2.5, and S/N ratios of single-base-mismatch combinations between probes and target DNAs were in the range from 1.0 to 1.2, respectively. The 5'SH control probe was used as control for T481C and G590A polymorphisms and the 3'SH control probe was used as control for G857A polymorphism.

The typing results of 38 samples with the automated system are shown in **Fig. 5 (B)** as a scatter chart. For each probe, four electrodes were assigned for validation and an average of I_{pa} from the four electrodes was adopted for genotyping analysis. An average of CV values of four electrodes on the control probes (5'SH and 3'SH) was 4.4%. Similarly, an average of CV values of four electrodes on the perfect-match-combinations between probes and target DNAs was 2.7%, and on the single-base-mismatch combinations

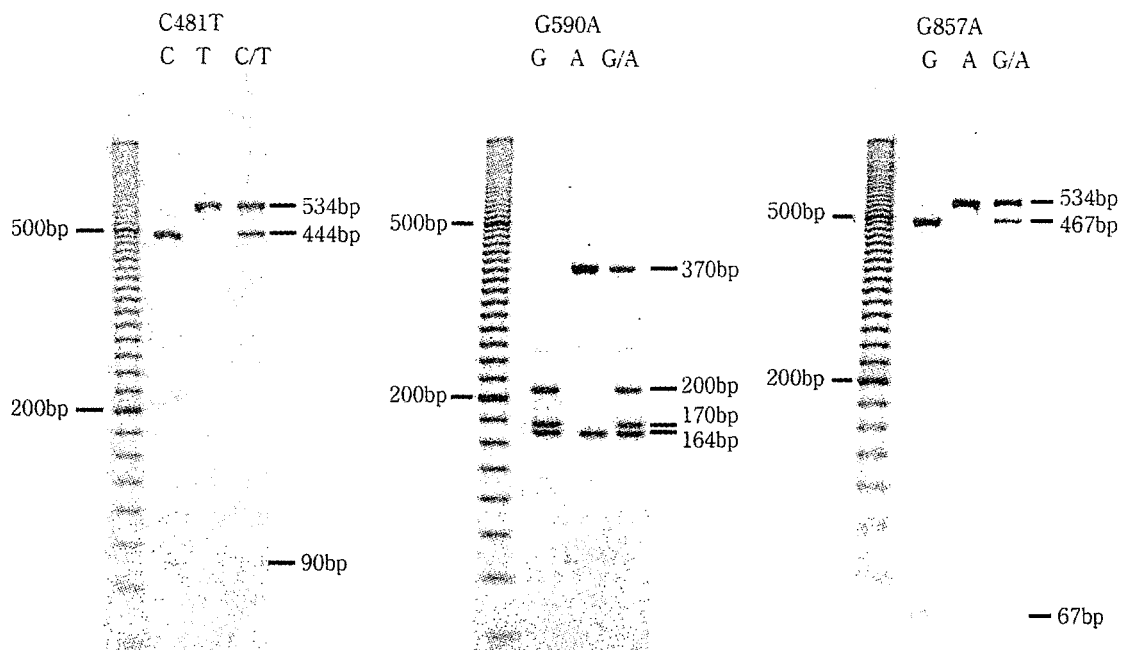


Figure 3 Results of PCR-RFLP analysis for NAT2 3SNPs. A marker is a 20bp DNA ladder (TAKARA Bio, Shiga, Japan).

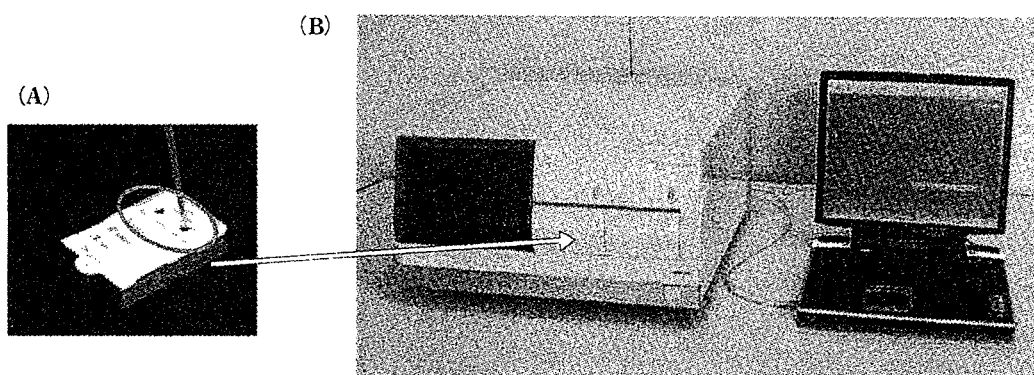


Figure 4 DNA chip cassette and automated DNA detection system.

(A) DNA chip cassette: DNA chip cassette includes the probe-immobilized-DNA chip substrate. The size is 30mm × 50mm × 6mm.

(B) Automated DNA detection system: The automated system has a space that stores reagents, such as 0.2 × SSC washing buffer and Hoechst33258 solution. Using two cassettes, simultaneous measurement is possible. The size is 450mm × 450mm × 200mm.

between probes and target DNAs was 4.2%. The distribution of each genotype of 3SNPs was clearly distinguished. The genotyping results by the detection system were all consistent with those by PCR-RFLP analysis. The turnaround time for genotyping with this system was 90 min.

IV. Discussion

In this study, NAT2 3SNPs genotyping was performed using the detection system that automatically performs the process after hybridization. As a result of genotyping of 38 samples, the feasibility of the automated system and that of the SNP genotyping algorithm were verified.

In Fig. 5 (B), there is wide variation among samples with an identical genotype. A difference of amplifi-

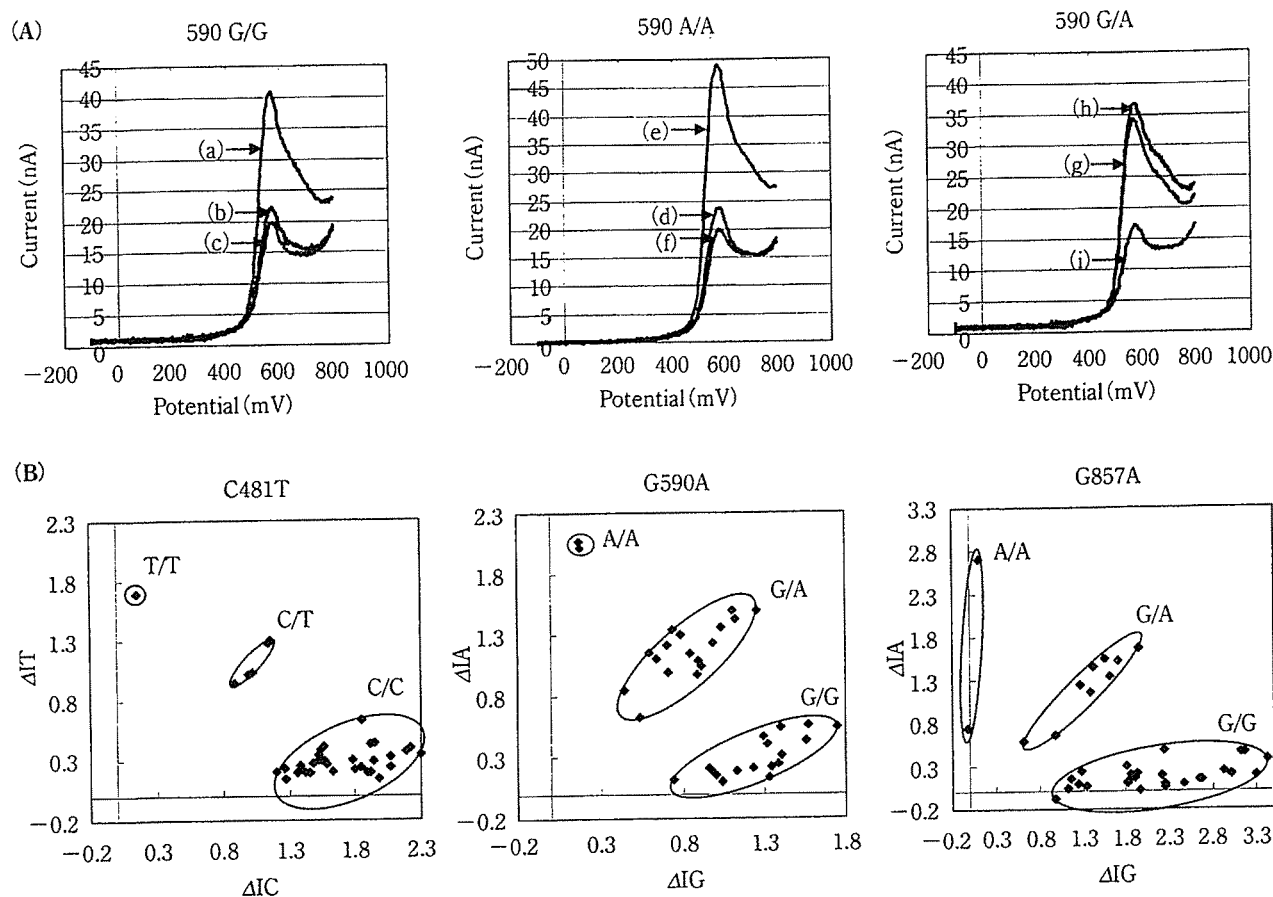


Figure 5 The electrochemical detection results of NAT2 3SNPs analyzed with the automated system.

(A) Voltammograms for G590A polymorphisms

(B) Typing results of 38 samples: IW: A average of I_{pa} values on wild probe, IM: A average of I_{pa} values on mutant probe, IB: A average of I_{pa} values on control probe

It is referred to as $\Delta IW = IW - IB$, $\Delta IM = IM - IB$. X-axis: $\Delta IW/IB$, Y-axis: $\Delta IM/IB$

cation efficiency by each human genome and a difference of treatment efficiency of double-stranded DNAs by T7 gene6 exonuclease are speculated as reasons. In particular, the treatment by the T7 gene6 exonuclease might be unstable and excessive digestion of DNA might occur. To solve such problem, we are now examining the combination of the LAMP¹⁶⁾ method, which is a novel technique for the amplification of specific DNA sequences under isothermal condition and contains the single-stranded DNAs in the amplification products.

Some studies on developments in electrochemical-based DNA or SNP detection technologies have been published. However, most of these studies can be categorized as fundamental verification steps because they use synthesized short oligonucleotides as the targets. There are only a few studies involving simultaneous genotyping of patient blood samples by use of the electrochemical DNA chip strategy. In these studies, the sandwich hybridization method using a DNA capture probe, a signaling probe, and a single-stranded target DNA has been reported⁴⁾. This is similar to our method, but it is disadvantageous that the ferrocene modification to the signaling probe is essential. On the other hand, the modification step is unnecessary and the procedures are very simple in the case of our method. Moreover, the Hoechst 33258 is available commercially and is cheap.

The DNA chip used in this study had 40 working electrodes. And the diameter of the electrode was 200 μm . The number of electrodes can be increased and the diameter can be changed according to the applications.

Moreover, we are now developing a fully automated system containing the DNA extraction and amplification. The fully automated system can detect DNAs from samples such as blood, mucous, and hair roots. Therefore, use of the system will not be restricted to technicians, but anyone will be able to use the system anywhere. We expect that the electrochemical DNA chip and the automated system will contribute to personalized medicine in the future.

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