DETECTION OF CLINICALLY IMPORTANT SNPS IN TPMT AND ABCC4 GENES BY THE WORLD’S FASTEST SNP-TYPING METHOD “SmartAmp”

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[Purpose] Thiopurines are effective anticancer agents and immunosuppressants. However, intracellular accumulation of their active metabolites, e.g., 6-TGN, causes dose-limiting hematopoietic toxicity. Since genetic polymorphisms in TPMT and ABCC4 genes have been suggested to exacerbate thiopurine toxicity, we aimed to develop a rapid genotyping method for detecting the genetic polymorphisms resulting in impairments of TPMT or ABCC4 function.

[Methods] To gain insight into the relationship between the non-functional alleles and the extent of thiopurine toxicity, we designed SmartAmp primers to detect the SNP 2269G>A in ABCC4 gene as well as TPMT*3A, *3B, and *3C.

[Results and Discussion] We have successfully developed SmartAmp primers for genotyping of these drug metabolizing enzyme and transporter that are critically involved in thiopurine-induced adverse reactions. By using genomic DNA samples, we have examined these SmartAmp primers in terms of detecting SNP 2269G>A in ABCC4 gene as well as TPMT*3A, *3B, and *3C. Furthermore, genotyping of SNP 2269G>A in ABCC4 gene was further examined by using blood samples from Japanese volunteers. The allele frequency of this SNP is higher in Asians than Caucasians. All the SmartAmp primers developed have been proven to accurately detect and discriminate all possible homozygotes and heterozygotes of the SNPs we tested. Thus, we will shortly implement clinical studies using our SmartAmp primers.


THE IMPACT OF VKORC1 AND CYP2C9 GENETIC POLYMORPHISM ON WARFARIN RESPONSE IN INDONESIAN PATIENTS

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[Purpose] VKORC1 and CYP2C9 genetic polymorphisms play a central role in warfarin efficacy and toxicity. Distribution of VKORC1 and CYP2C9 genotypes differ from one population to others. To confirm the impact of these polymorphism to warfarin response, we evaluated the relationship between VKORC1 and CYP2C9 genotypes and warfarin response in Indonesian population.

[Methods] Genotyping assays of VKORC1 and CYP2C9 in 103 Indonesian healthy volunteers and 78 patients that treated with warfarin have been carried out by PCR-RFLP method. The concentration of R-warfarin and S-warfarin in plasma, and prothombine time expressed as international normalized ratio (PT-INR) were used as warfarin response parameters. The data were assessed by multivariate analysis method.

[Results and Discussion] The PT-INR was higher in VKORC1-1639 GG genotype patients compared to those with GA and AA genotype (p<0.005), and higher in patients with CYP2C9 *1/*1 genotype compare to those with wild-type (p<0.05). S-warfarin concentration was higher in CYP2C9 heterotype (*1/*3) patients compared to those with wild-type (p<0.02).

[Conclusions] Genetic polymorphisms of VKORC1-1639 G>A and CYP2C9 gave a significant impact to PT-INR and S-warfarin concentration variability as parameters of warfarin response in Indonesian patients.