論文の内容の要旨

論文題目　**Identification of genetic determinants for warfarin responsiveness**

和訳　ワーファリン薬剤感受性遺伝子の同定

指導教員　中村祐輔教授

東京大学大学院医学系研究科

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氏名　**CHA　PEI　CHIENG**（チャ　ペイ　チェン）

Warfarin is a commonly-used anticoagulant whose dose needs to be determined for each individual patient owing to large inter-individual variability in its therapeutic dose. Several clinical and genetic factors have been shown to influence warfarin dose, and these predictors include age, body size, co-administration of drugs and intake of vitamin K. Recently, several studies have disclosed that genetic variations in the *CYP2C9* and the *VKORC1* genes are associated with the effect of warfarin. Nevertheless, a large part of inter-individual variability in warfarin maintenance dose remains unexplained. Hence, uncovering additional predictors of the dose is critically important for the safer use of this drug. To identify genetic determinants of warfarin responsiveness in Japanese, I performed both a candidate gene study and a genome-wide association study (GWAS).

In the candidate gene study, I determined genetic variations in eight genes that were known to play important roles in the mode of action of warfarin, and examined their

associations with warfarin responsiveness. The eight genes include *GGCX, GAS6, PROC*, *PROS1*, *PROZ*, *PXR, CAR,* and *HNF4A,* encoding the gamma-glutamyl carboxylase, protein C, protein S, protein Z, growth-arrest specific 6, pregnane X receptor, constitutive androstane receptor, and hepatocyte nuclear factor 4 alpha, respectively. Among the eight genes, *GGCX*, a vitamin K-dependent (VKD) carboxylase, is involved in the activation of VKD proteins. By means of direct sequencing, an 18-kb genomic region containing the *GGCX* gene was analyzed in 96 Japanese subjects who received warfarin therapy. A total of 41 single nucleotide polymorphisms (SNPs), seven insertion/deletion polymorphisms, and a microsatellite polymorphism were identified. I compared the genotypes of these polymorphisms with the warfarin maintenance dose, but no significant associations were observed in the 96 subjects. In addition, I investigated a total of 80 functional SNPs and tagging SNPs (tSNPs) in the remaining seven candidate genes, namely, *PROC, PROS1, PROZ*, *GAS6, PXR*, *CAR,* and *HNF4A* in the 96 subjects. All tSNPs analyzed in this study were reported to have minor allele frequencies of greater than 10% in the Japanese HapMap database and covered most of haplotypes in linkage disequilibrium blocks encompassing the seven genes (R^2>0.8). In total, 14, 7, 9, 10, 5, 9, and 26 SNPs for each of the aforementioned genes were genotyped, respectively. Subsequent association studies revealed that none of the SNPs showed significant associations with warfarin responsiveness.

In the GWAS, I carried out genotyping of 1515 Japanese subjects including 811

whose therapeutic warfarin dose are ≤1mg/day and 704 whose therapeutic warfarin dose are ≥4 mg/day. Comparison of the genotypes between the two groups identified 18 SNPs that showed suggestive associations ($P < 1x10^{-5}$) with warfarin responsiveness. A subsequent replication study using an additional 444 Japanese subjects revealed that an SNP, rs2108622, in the *CYP4F2* was significantly associated with warfarin responsiveness ($P_{Combined} = 2.57 \times 10^{-8}$). The effect of SNP rs2108622 *(CYP4F2)* in the association could be masked by the stronger effects of age, body surface area (BSA), and SNPs in the *CYP2C9* and *VKORC1* genes that were reported to influence the warfarin dose of Japanese. In addition, detailed analysis of the data enabled me to estimate that a T-allele of rs2108622 would increase the warfarin maintenance dose by 0.23 mg/day. Furthermore, I found through a regression analysis that co-administration of amiodarone, an inhibitor of CYP2C9, also significantly influence therapeutic warfarin dose. On the basis of these findings, I developed a warfarin dosing algorithm that incorporated not only information on age, BSA, and genotypes of SNPs rs10509680 (*CYP2C9)* and rs9923231 *(VKORC1),* but also the status of amiodarone co-administration and genotypes of SNP rs2108622 *(CYP4F2).* This algorithm correctly predicted 43.4% of warfarin dose variability of the 444 subjects. Although the dosing algorithm is preliminary and subsequent evaluation of it in prospective study is necessary, the findings shown in this study have deepen our understanding on the pharmacogenetics of warfarin and paved a way to the safer use of the drug.